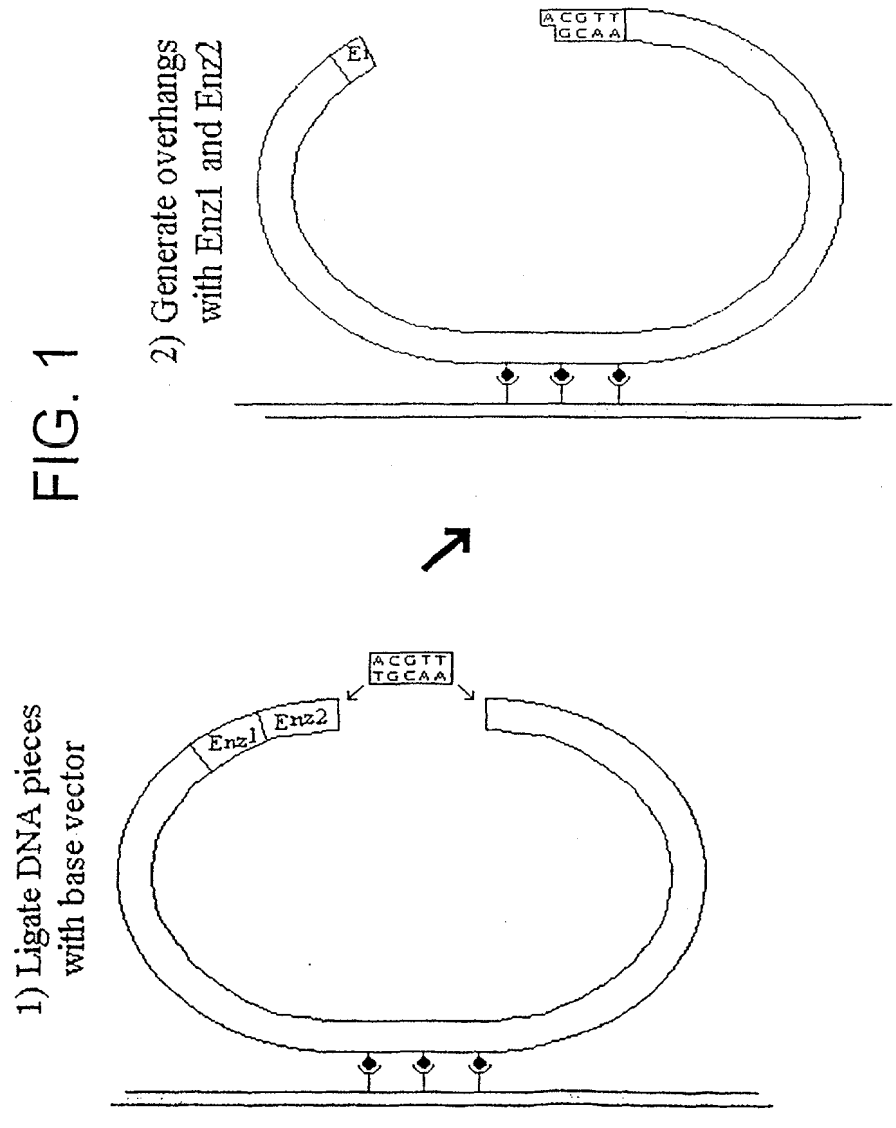
1) Ligate DNA pieces with base vector
ACGTT
TGCAA
Enz1
Enz2
2) Generate overhangs with Enz1 and Enz2
ACGTT
GCAA

FIG. 1

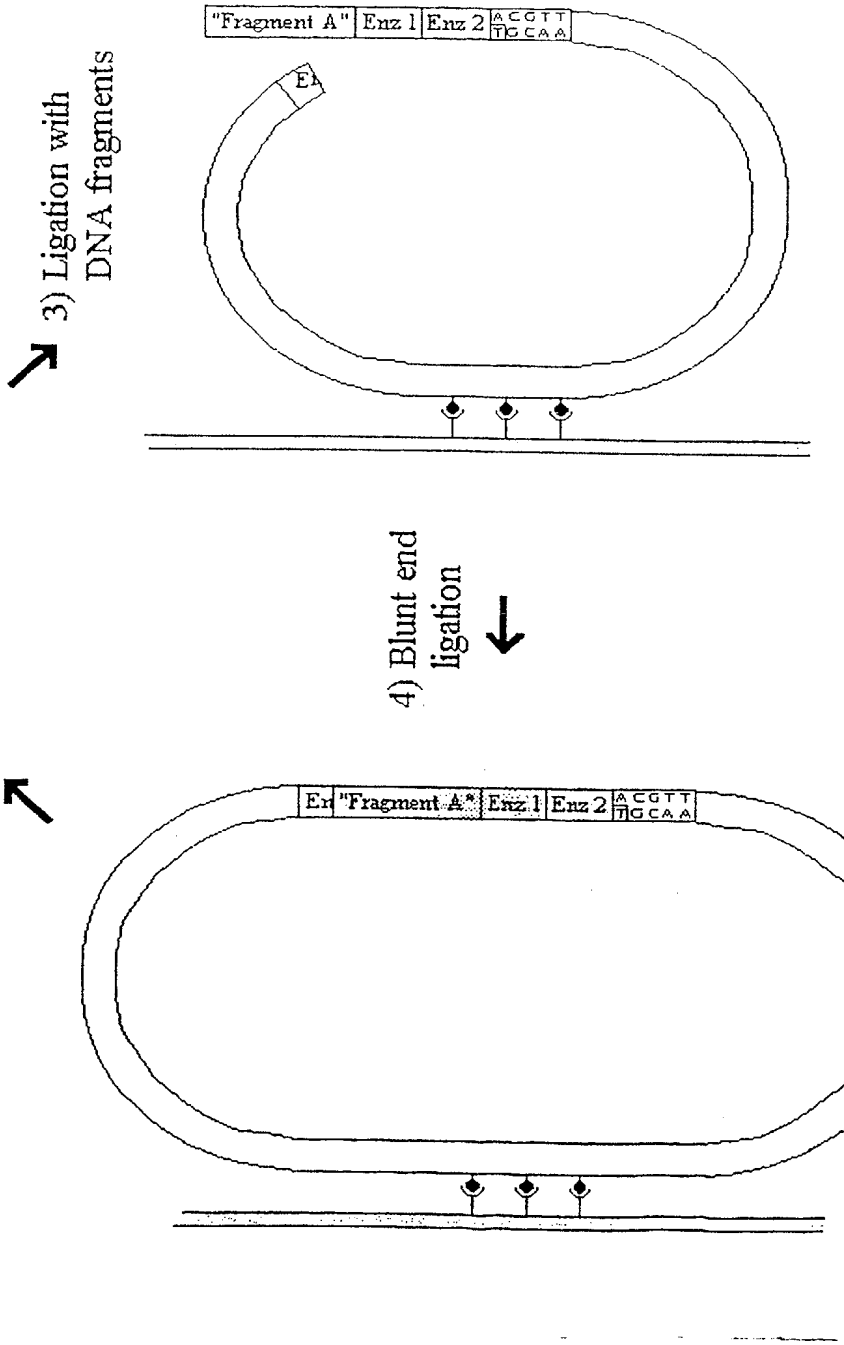
3) Ligation with DNA fragments
"Fragment A"
Enz 1
Enz 2
ACGTT
TGCAA
4) Blunt end ligation
"Fragment A*"
Enz 1
Enz 2
ACGTT
TGCAA

FIG. 1 CONT'D

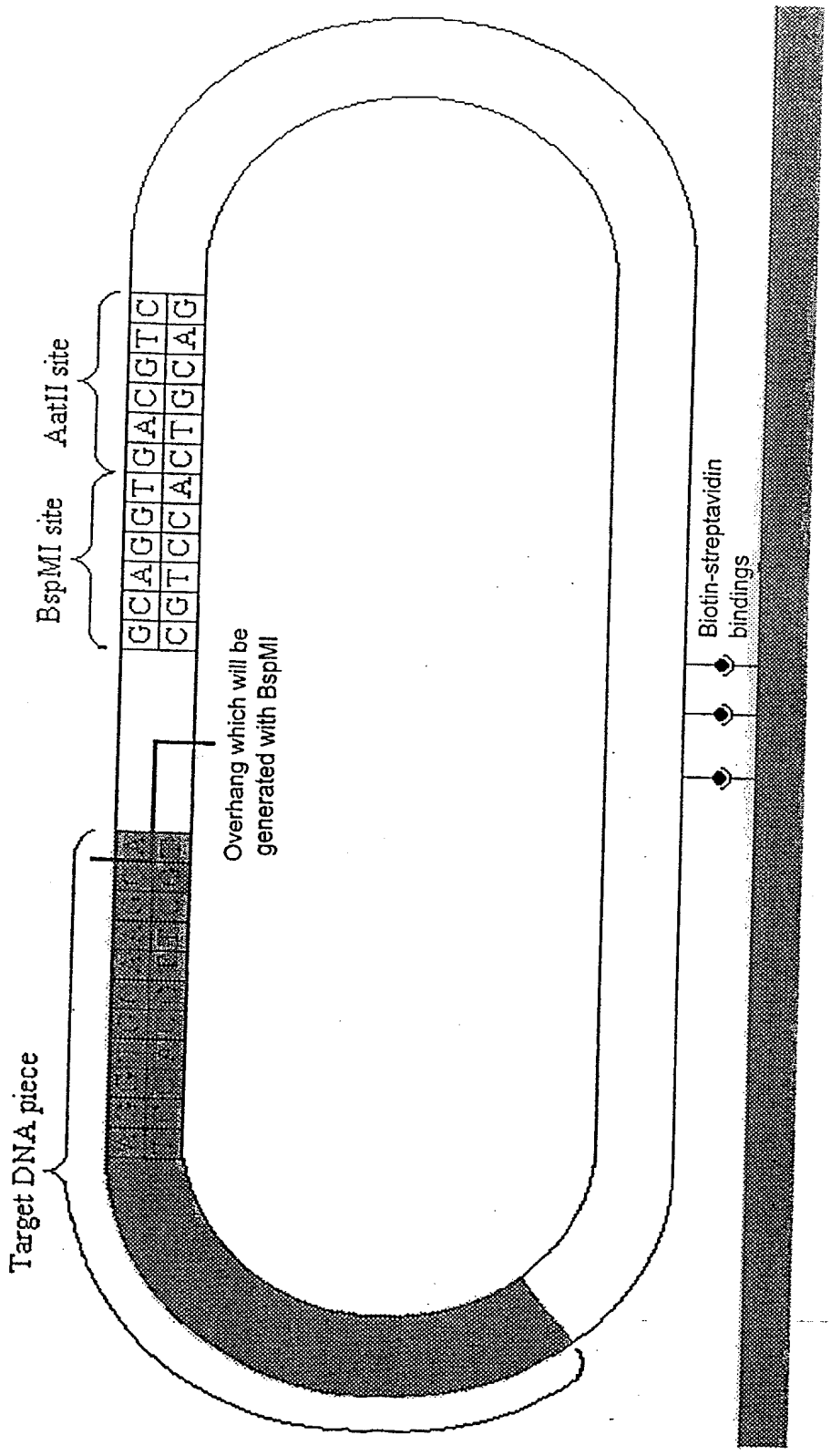
Target DNA piece
BspMI site
AatII site
G C A G G T G A C G T C
C G T C C A C T G C A G
Overhang which will be generated with BspMI
Biotin-streptavidin bindings

FIG. 2A

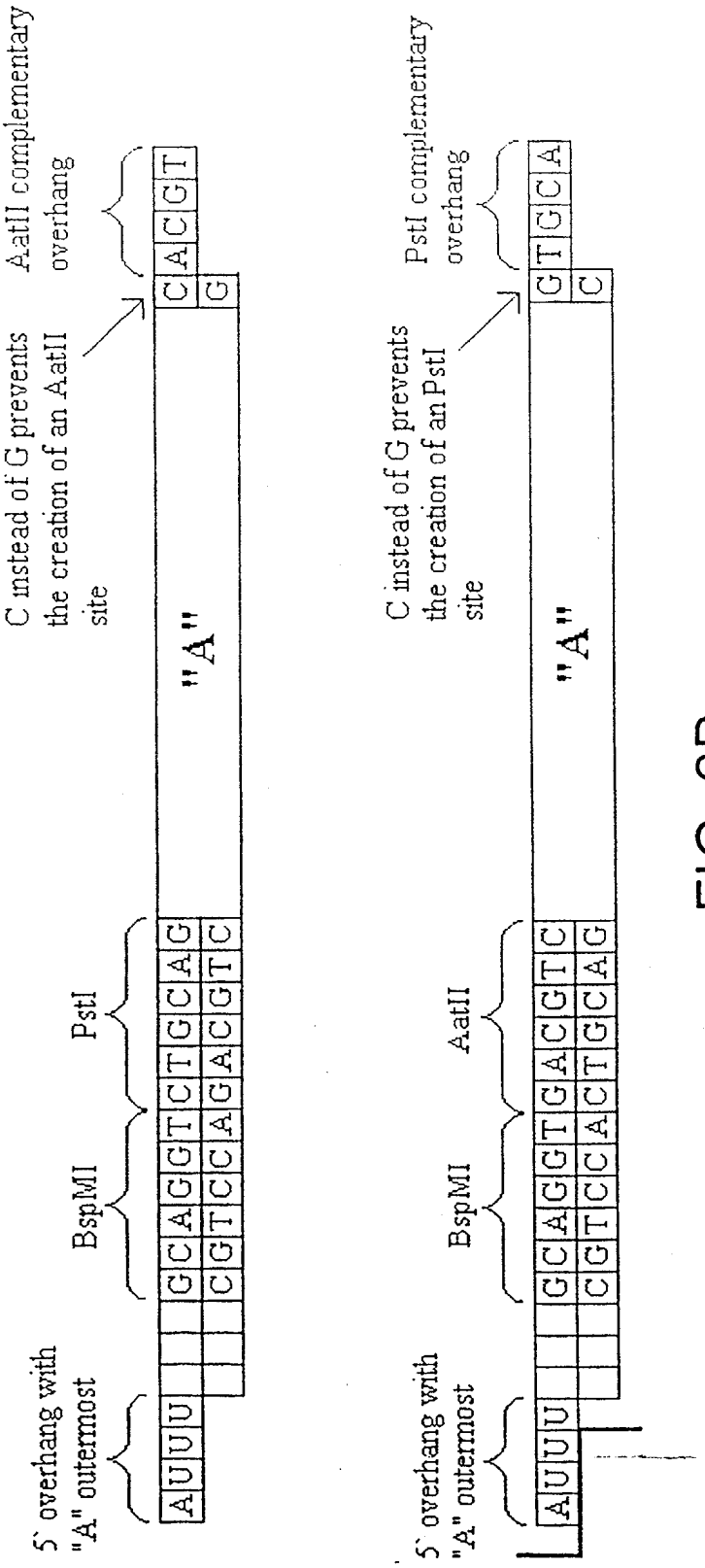
C instead of G prevents the creation of an AatII site
AatII complementary overhang
BspMI
PstI
5` overhang with "A" outermost
"A"
C instead of G prevents the creation of an PstI site
PstI complementary overhang
BspMI
AatII
5` overhang with "A" outermost
"A"

FIG. 2B

FIG. 2C


DNA piece that begins with adenine
BspMI
AatII
Biotin-streptavidin bonds
Cut with BspMI
Ligate with an excess of adapter-A1, -C1, -G1 and -T1
AatII overhang
"A"
PstI
Adapter-A1
Only adapter-A will be ligated to DNA pieces that contains T in the innermost position

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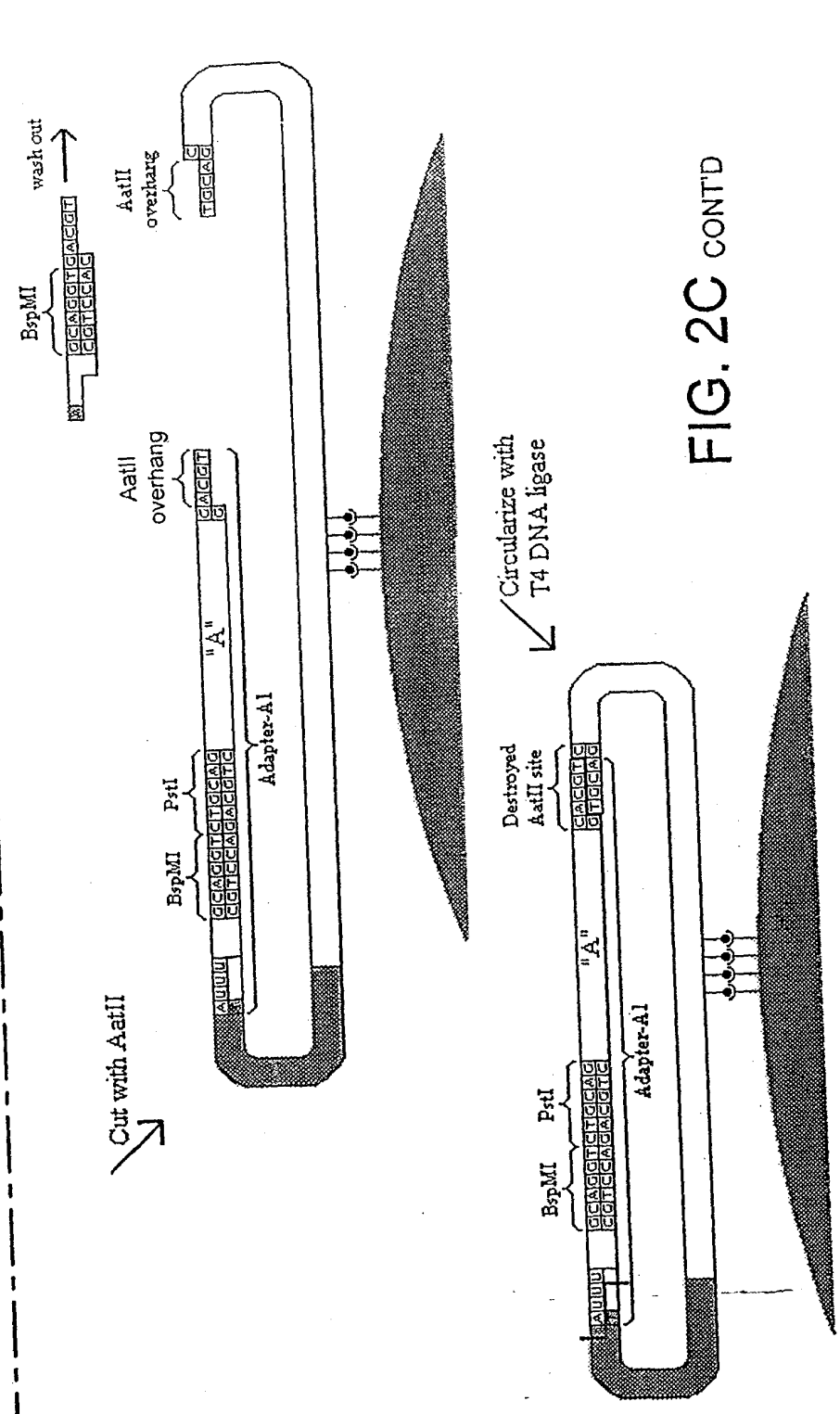



FIG. 2C CONT'D

SUBSTITUTE SHEET (RULE 26)

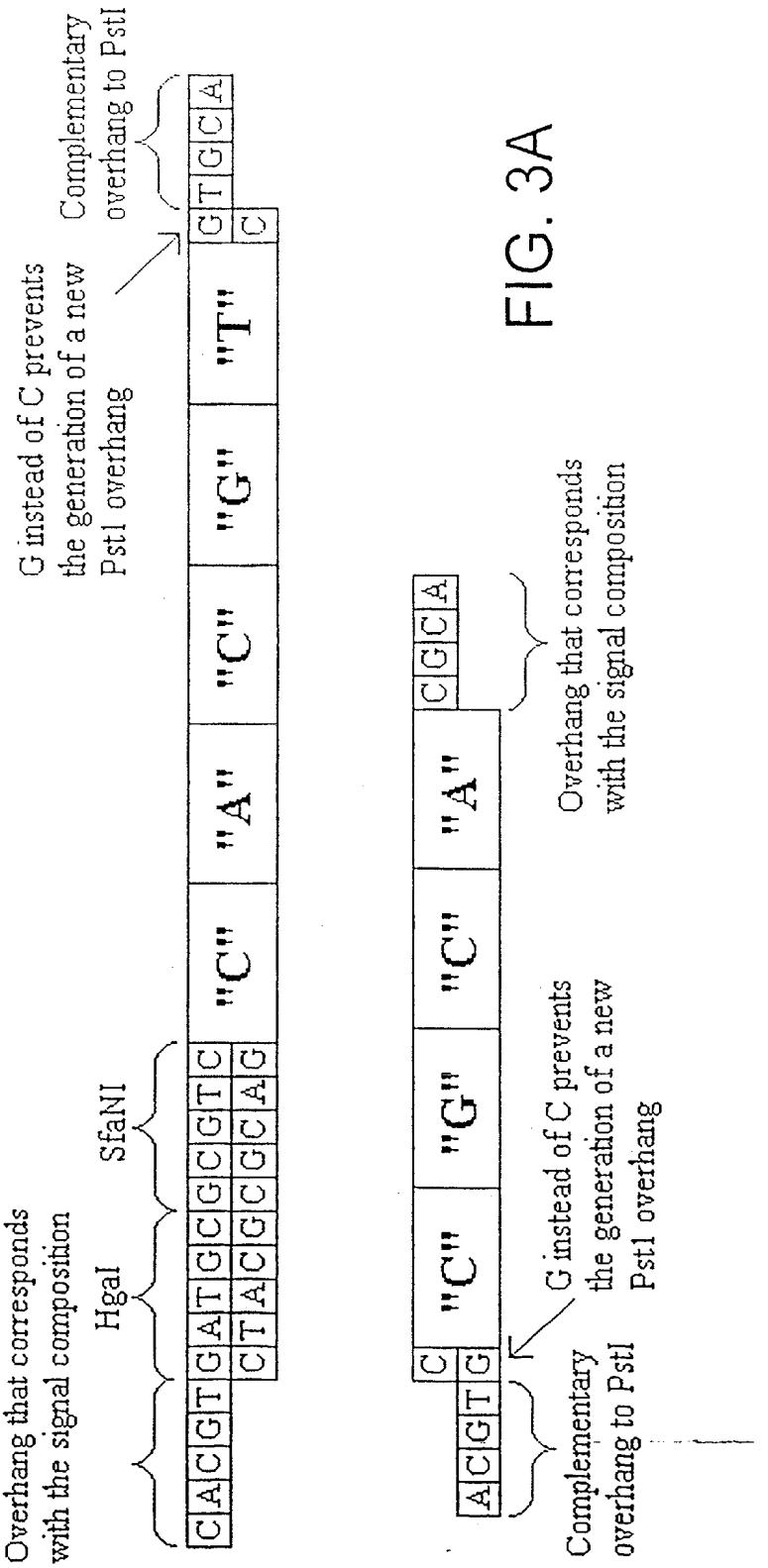
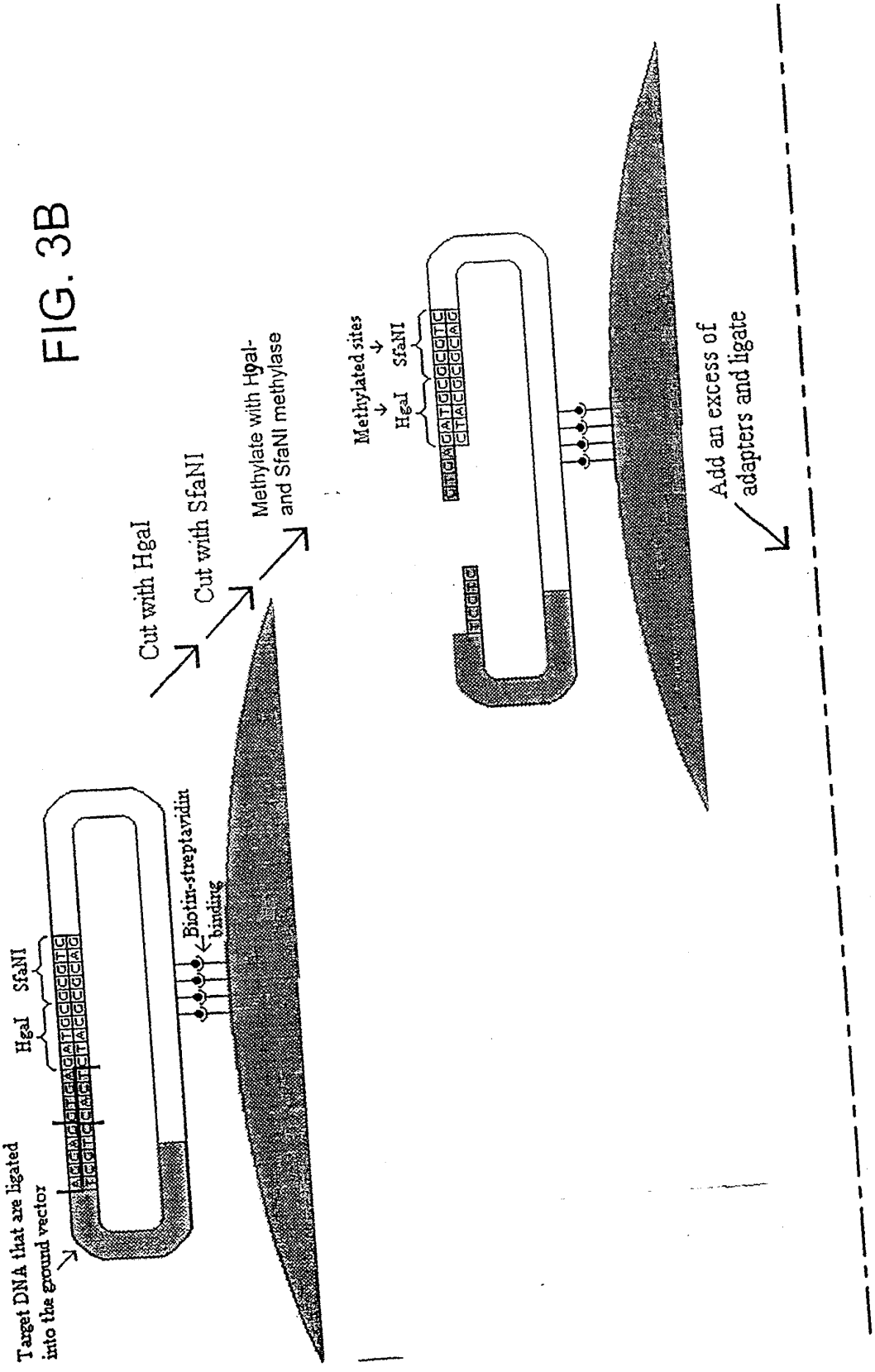

Overhang that corresponds with the signal composition
HgaI
SfaNI
C A C G T G A T G C G C G T C
C T A C G C G C A G
"C" "A" "C" "G" "T"
G instead of C prevents the generation of a new PstI overhang
Complementary overhang to PstI
A C G T G
C "C" "G" "C" "A"
C G C A
Overhang that corresponds with the signal composition
G instead of C prevents the generation of a new PstI overhang
Complementary overhang to PstI

FIG. 3A

FIG. 3B


Target DNA that are ligated into the ground vector
HgaI
SfaNI
Biotin-streptavidin binding
Cut with HgaI
Cut with SfaNI
Methylate with HgaI- and SfaNI methylase
Methylated sites
HgaI
SfaNI
Add an excess of adapters and ligate

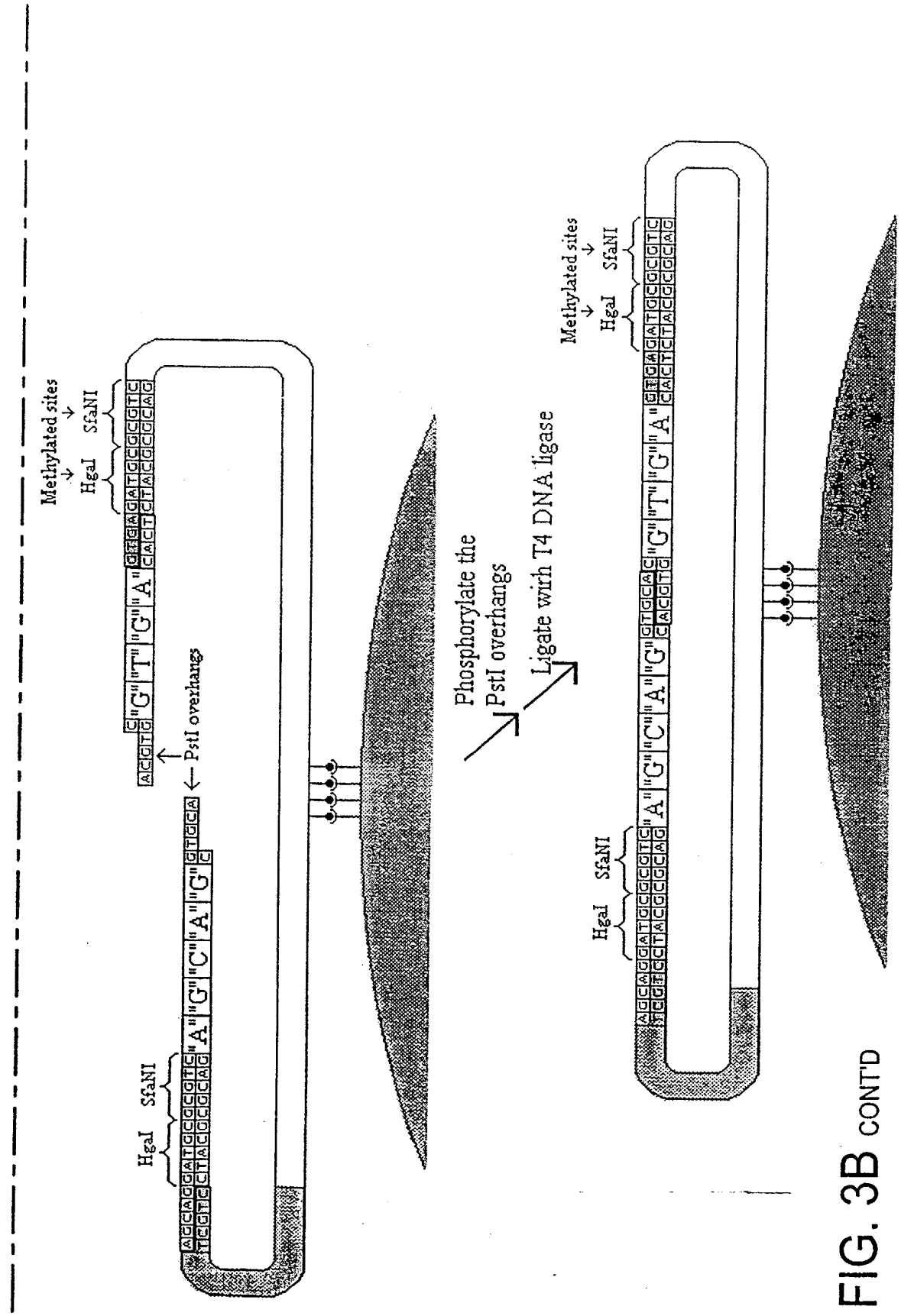

Methylated sites
HgaI SfaNI
PstI overhangs
Phosphorylate the PstI overhangs
Ligate with T4 DNA ligase
Methylated sites
HgaI SfaNI
HgaI SfaNI

FIG. 3B CONT'D

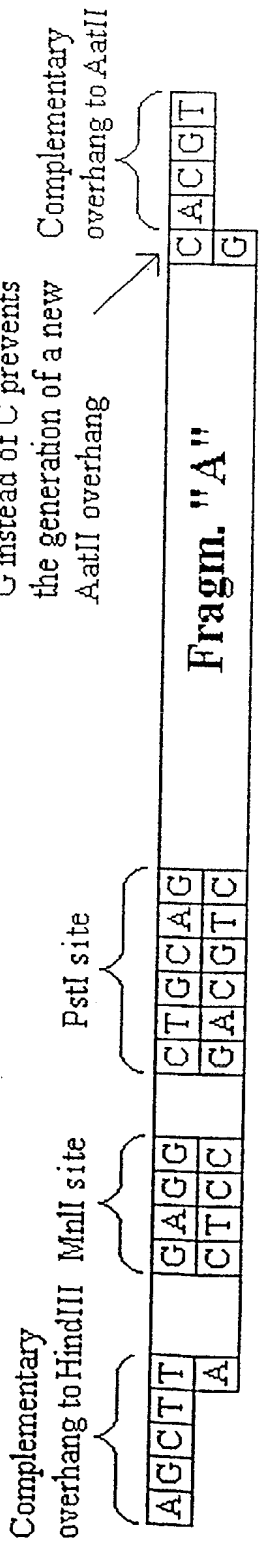

Complementary overhang to HindIII
MnlI site
PstI site
G instead of C prevents the generation of a new AatII overhang
Complementary overhang to AatII
Fragm. "A"

FIG. 4A

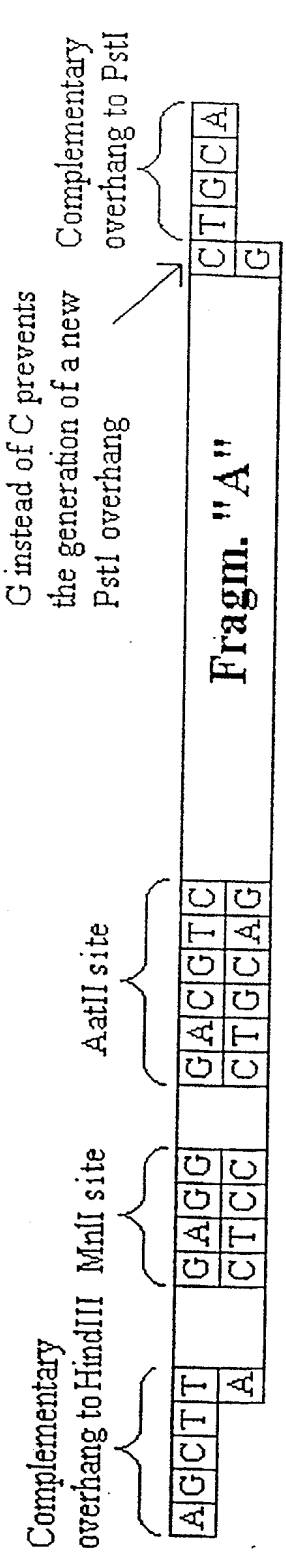

Complementary overhang to HindIII
MnlI site
AatII site
G instead of C prevents the generation of a new PstI overhang
Complementary overhang to PstI
Fragm. "A"
Hind III site that miss one -A
MnlI site

FIG. 4B

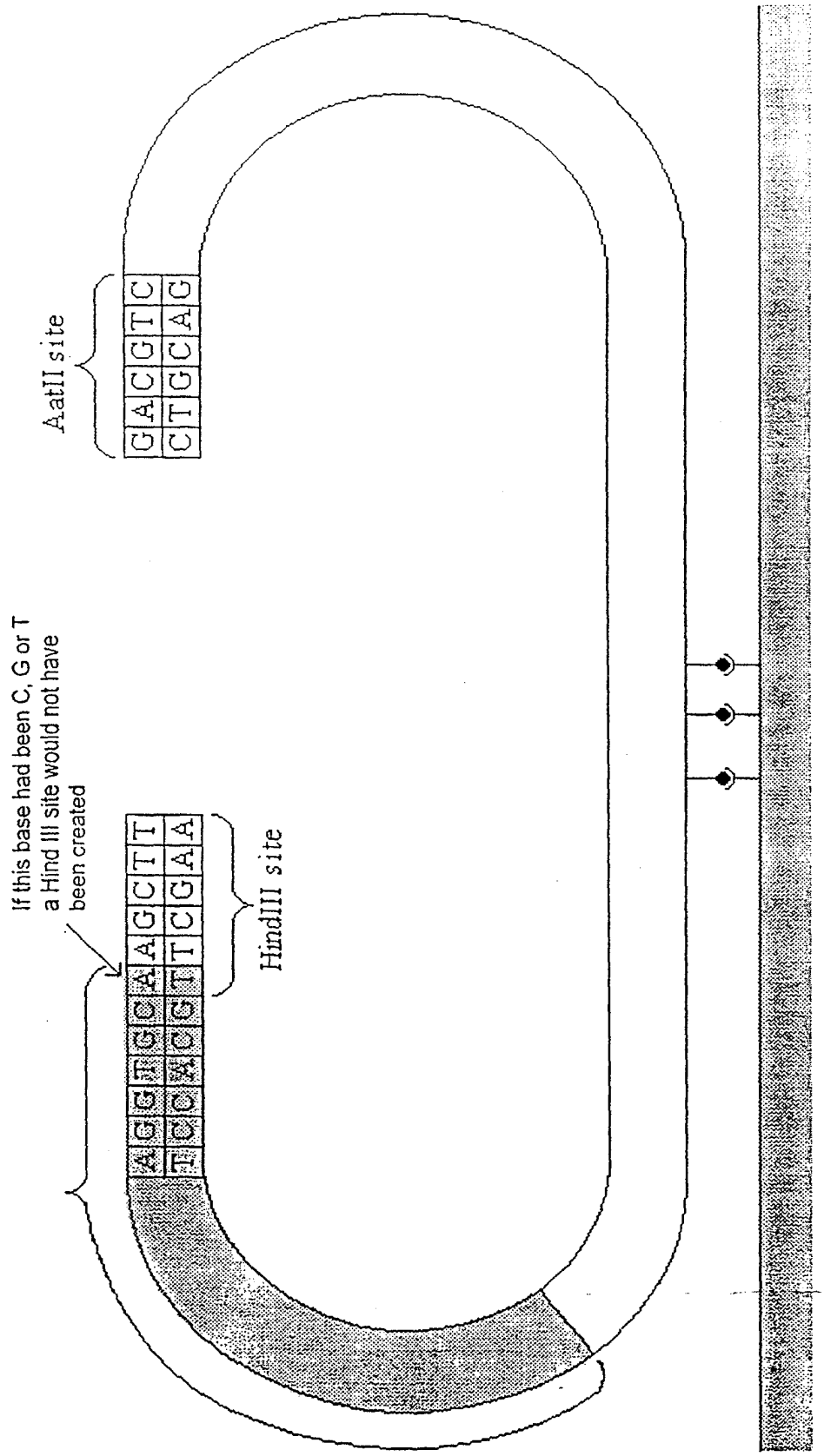

AatII site
If this base had been C, G or T a Hind III site would not have been created
HindIII site

FIG. 4C

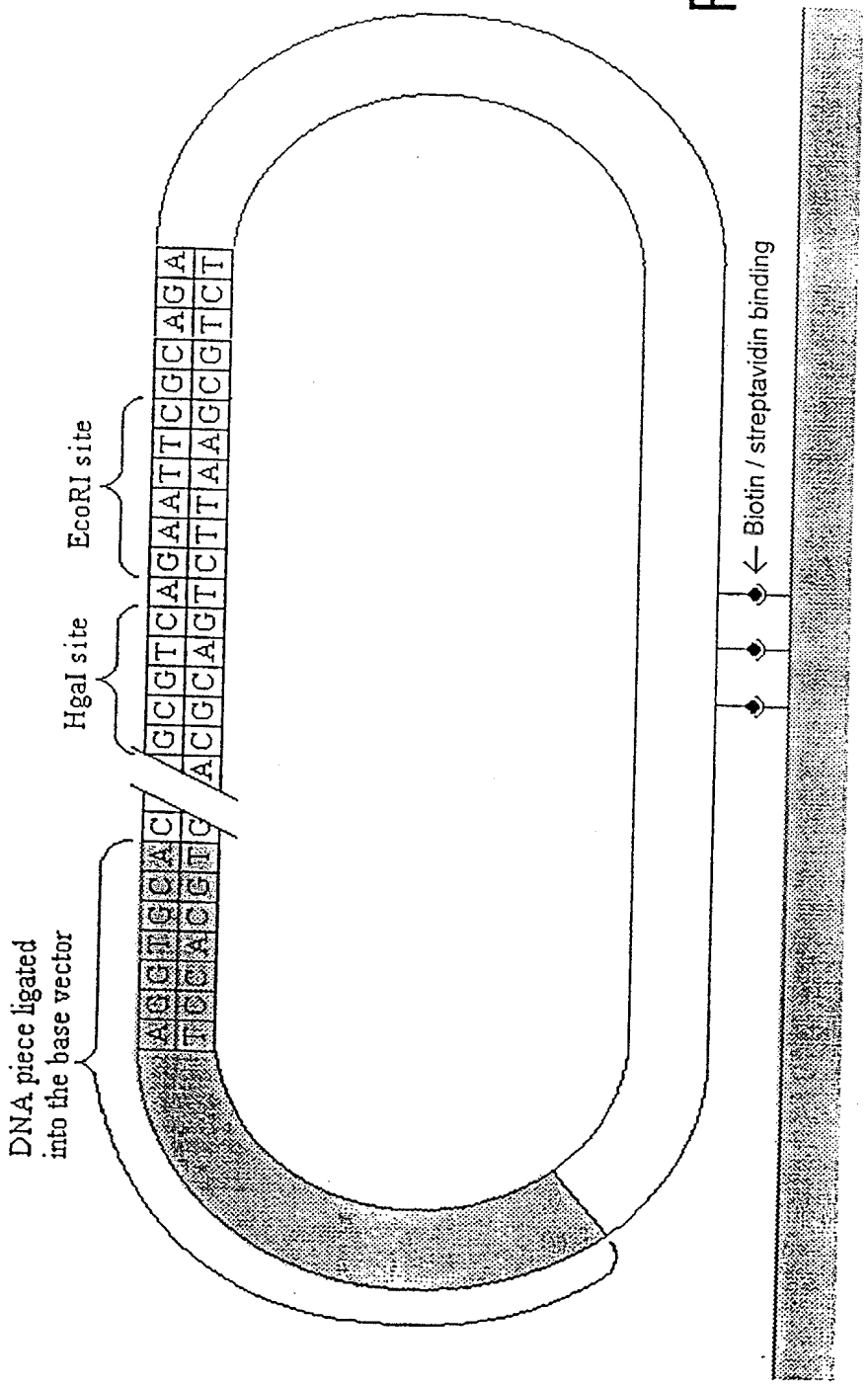

DNA piece ligated into the base vector
HgaI site
EcoRI site
← Biotin / streptavidin binding

FIG. 5A


Overhang with universal nucleotides
HgaI site
PstI site
G instead of C prevents the generation of a new AatII overhang
Complementary overhang to AatII
Fragm. "A"

FIG. 5B

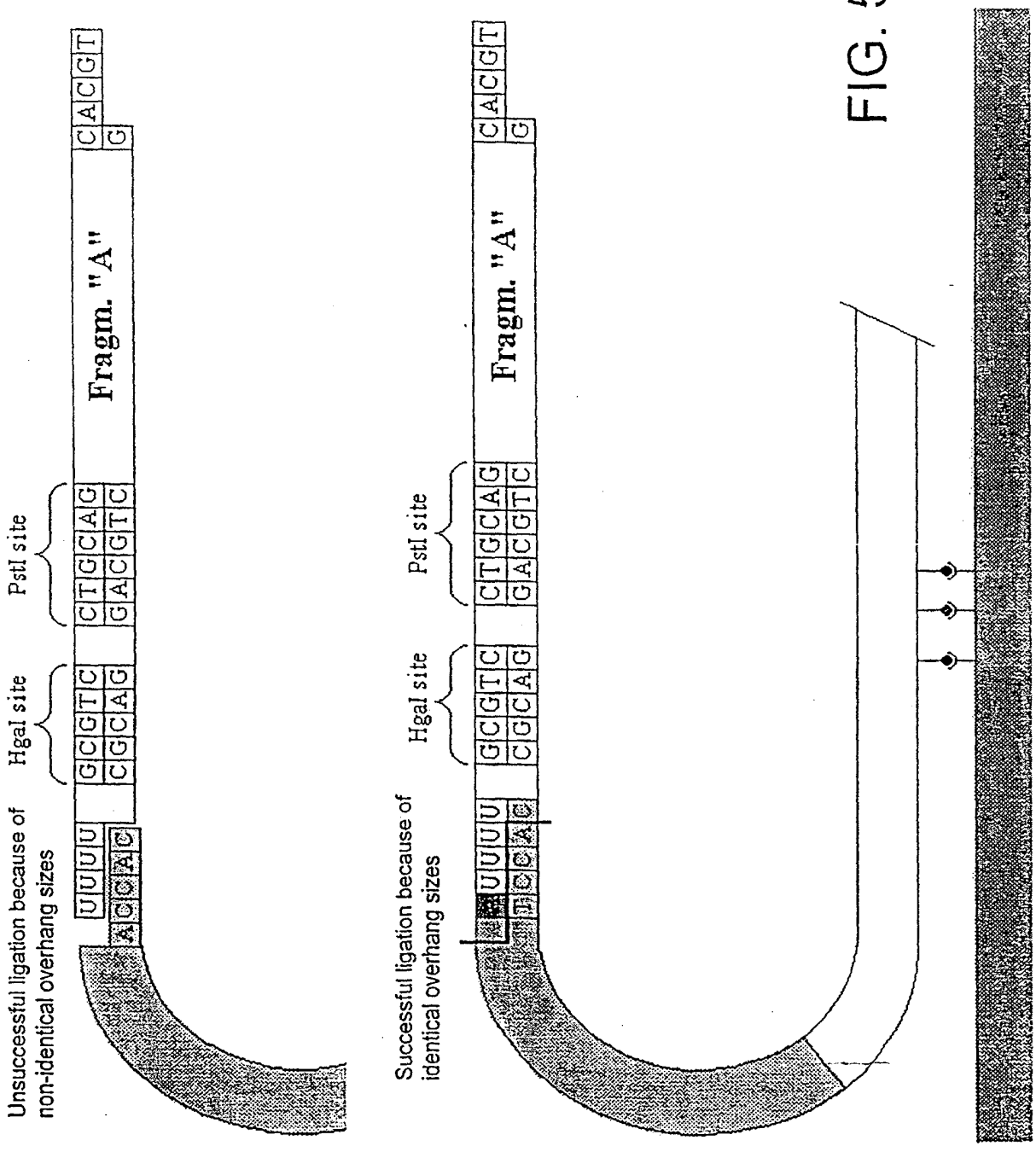

Unsuccessful ligation because of non-identical overhang sizes
HgaI site
PstI site
Fragm. "A"
Successful ligation because of identical overhang sizes
HgaI site
PstI site
Fragm. "A"

FIG. 5C

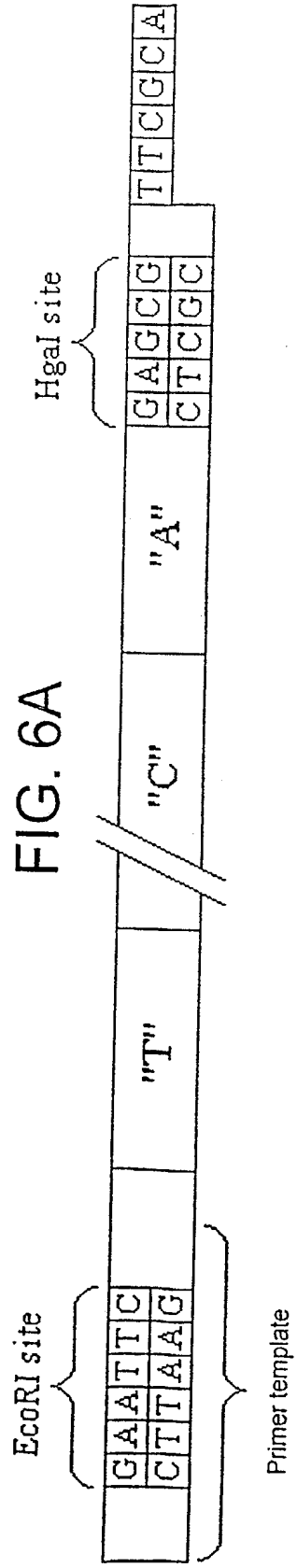

EcoRI site
HgaI site
"T"
"C"
"A"
Primer template

FIG. 6A

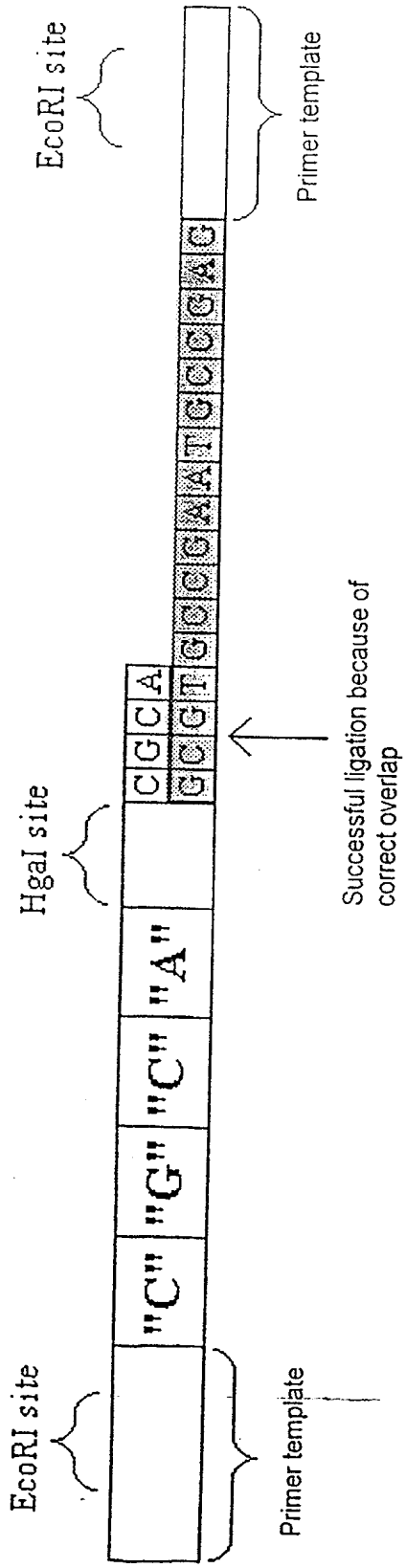

Primer-template
DNA piece
Adapter
EcoRI site
HgaI site
"C"
"G"
"C"
"A"
Successful ligation because of correct overlap
Primer template
Primer template

FIG. 6B

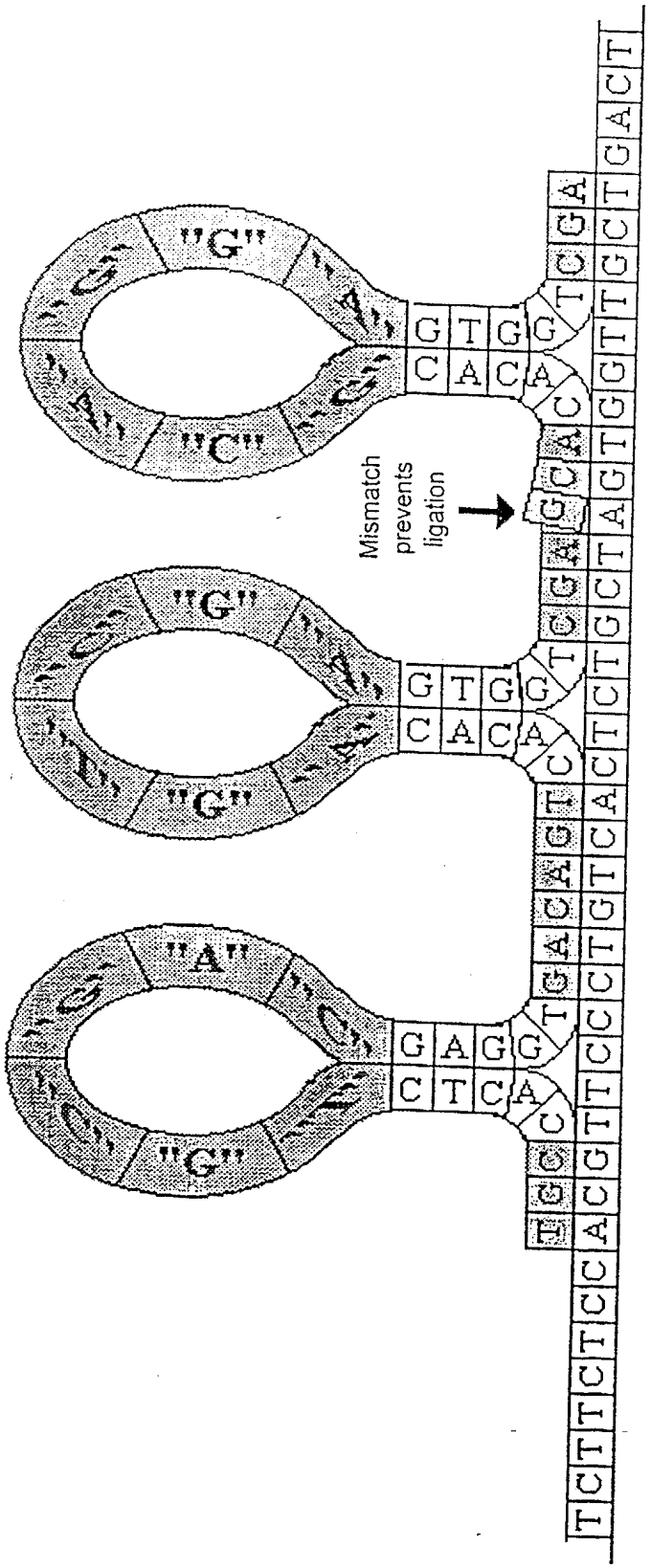



FIG. 7

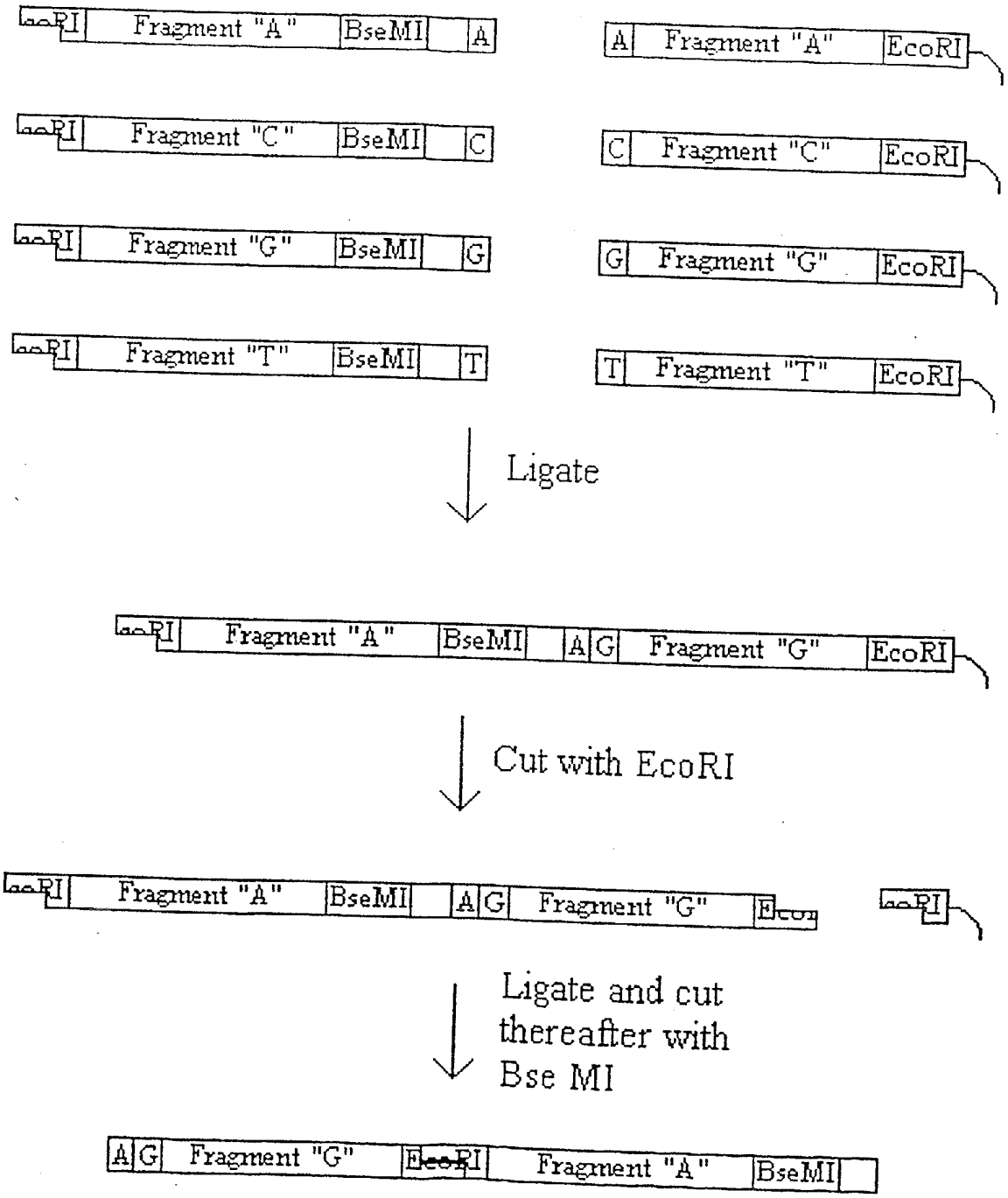

EcoRI
Fragment "A"
BseMI
A
Fragment "A"
EcoRI
EcoRI
Fragment "C"
BseMI
C
C
Fragment "C"
EcoRI
EcoRI
Fragment "G"
BseMI
G
G
Fragment "G"
EcoRI
EcoRI
Fragment "T"
BseMI
T
T
Fragment "T"
EcoRI
Ligate
EcoRI
Fragment "A"
BseMI
A
G
Fragment "G"
EcoRI
Cut with EcoRI
EcoRI
Fragment "A"
BseMI
A
G
Fragment "G"
EcoRI
Ligate and cut
thereafter with
Bse MI
A
G
Fragment "G"
EcoRI
Fragment "A"
BseMI

FIG. 8

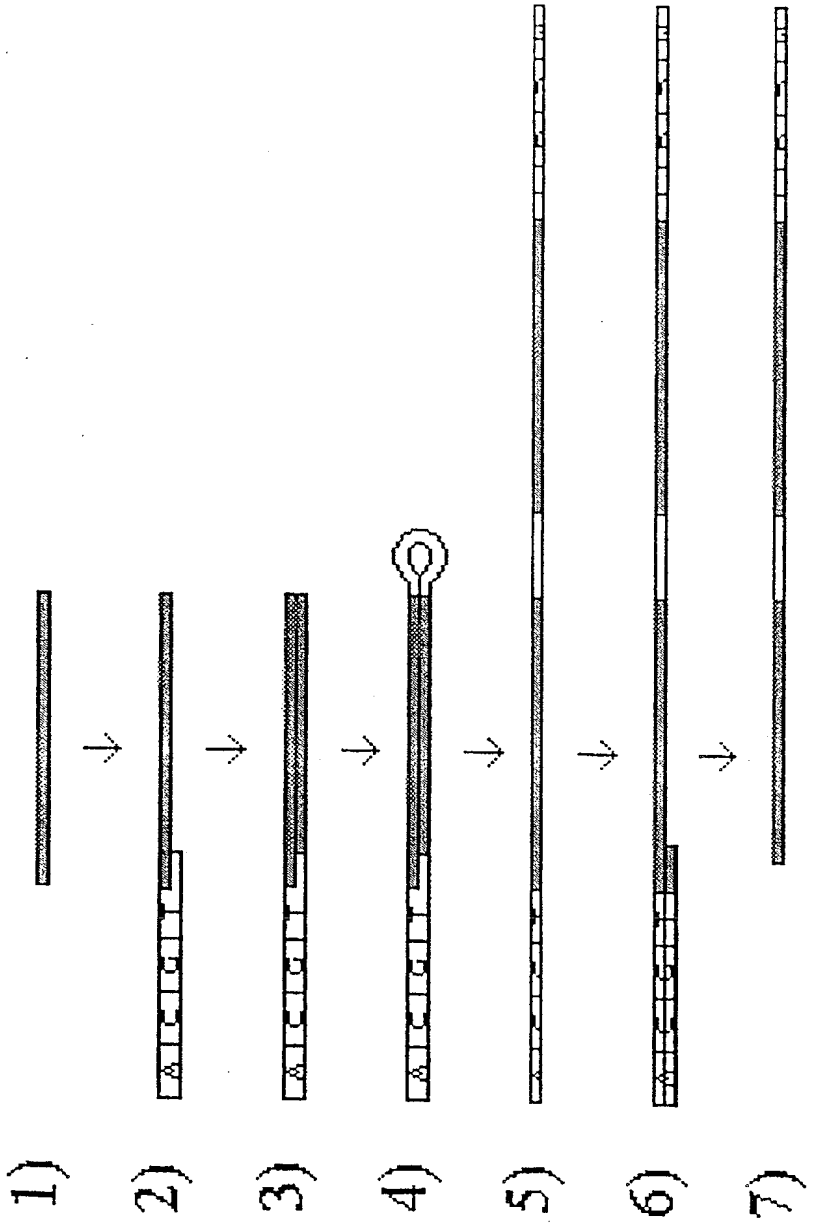
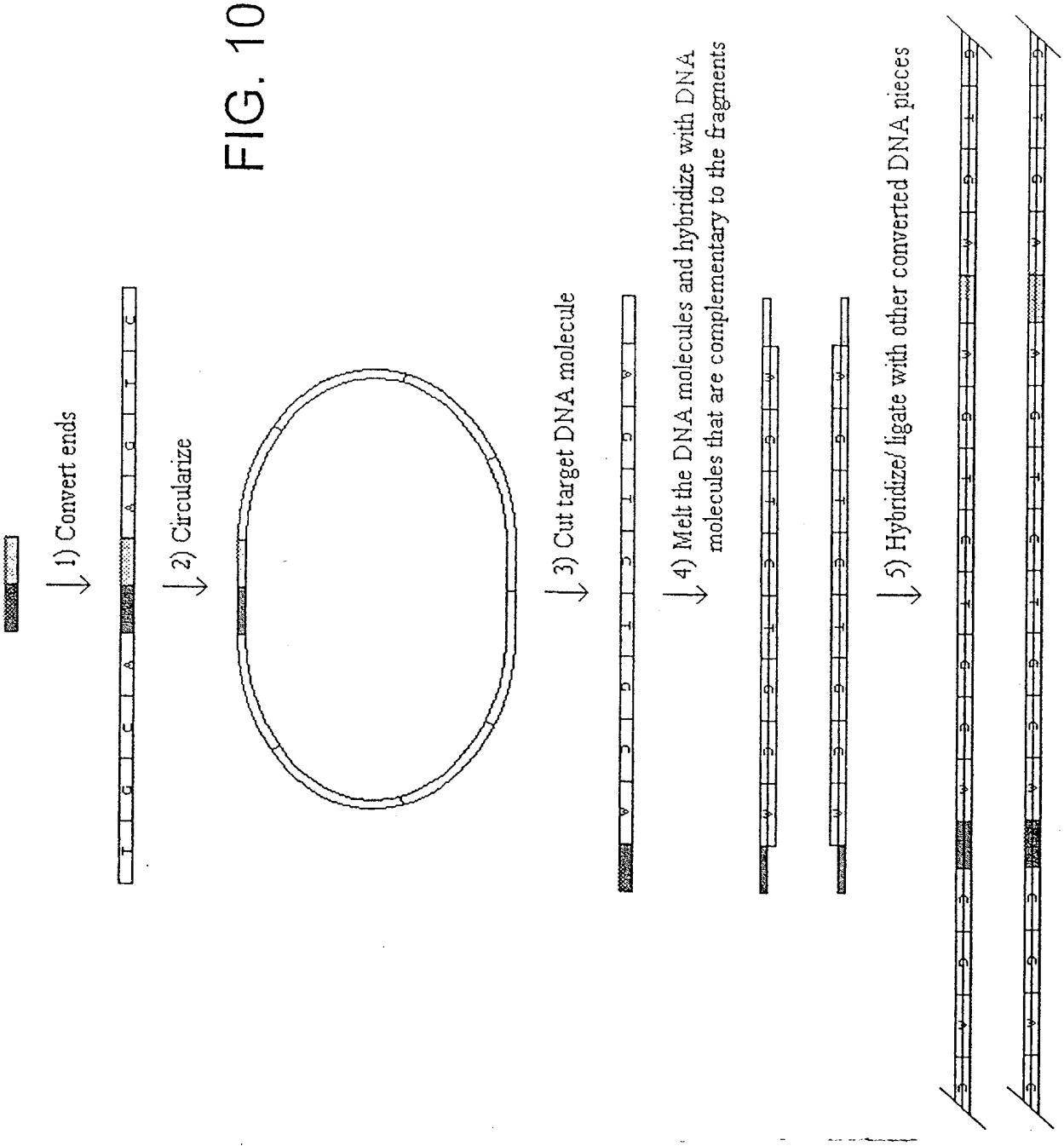



FIG. 9

FIG. 10


1) Convert ends
2) Circularize
3) Cut target DNA molecule
4) Melt the DNA molecules and hybridize with DNA molecules that are complementary to the fragments
5) Hybridize/ ligate with other converted DNA pieces

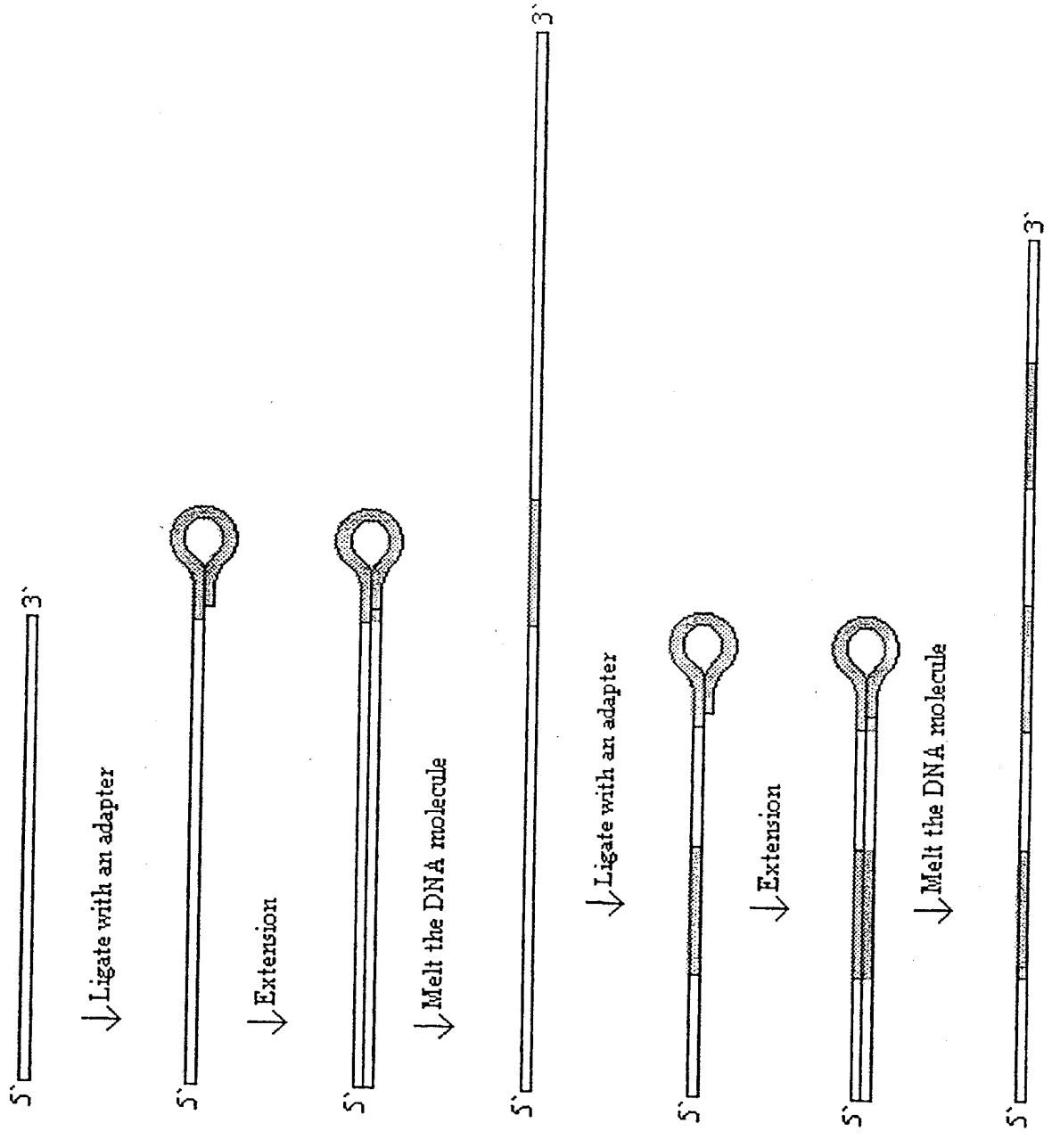

5`
3`
Ligate with an adapter
5`
Extension
5`
Melt the DNA molecule
5`
3`
Ligate with an adapter
5`
Extension
5`
Melt the DNA molecule
5`
3`

FIG. 11

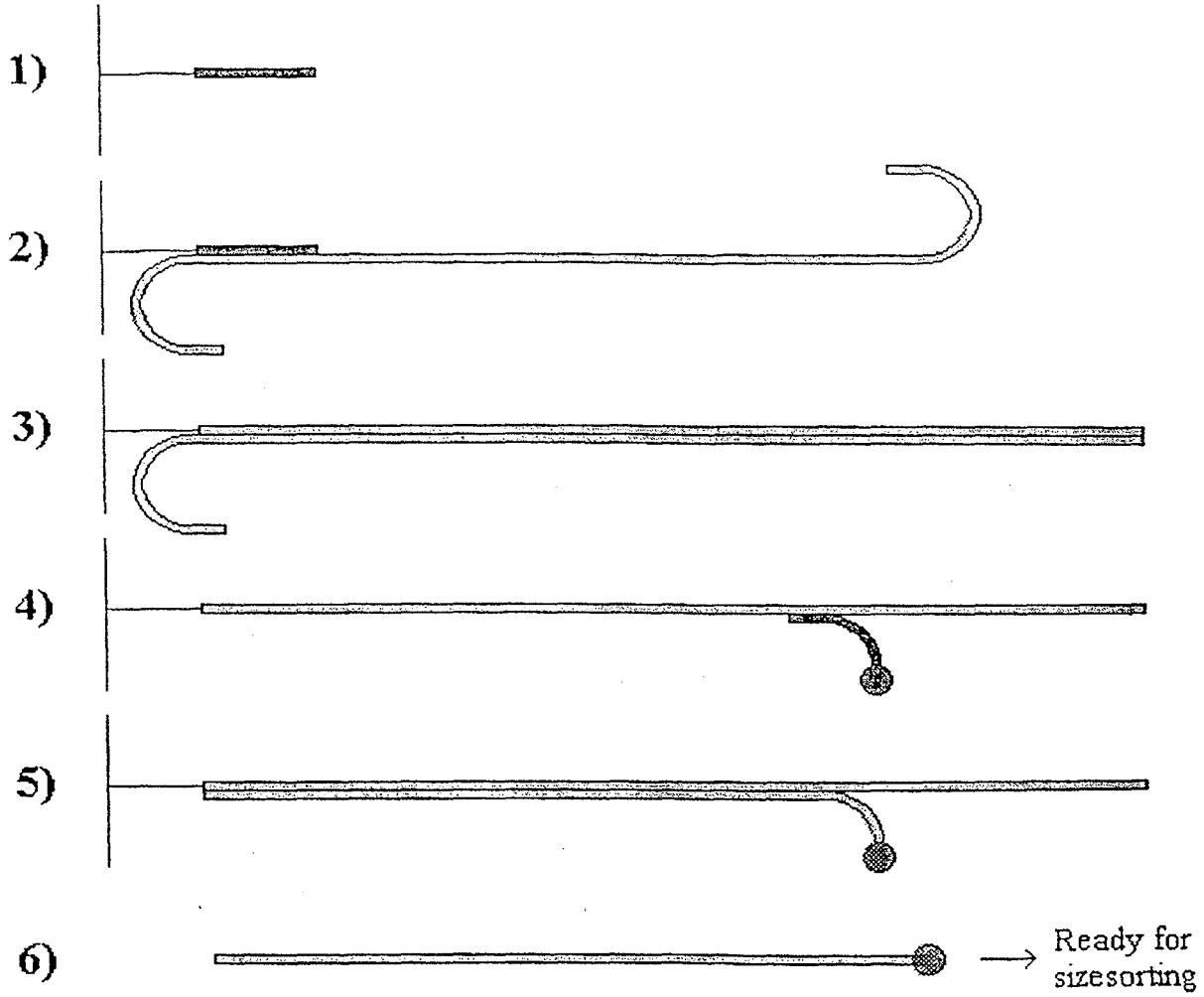


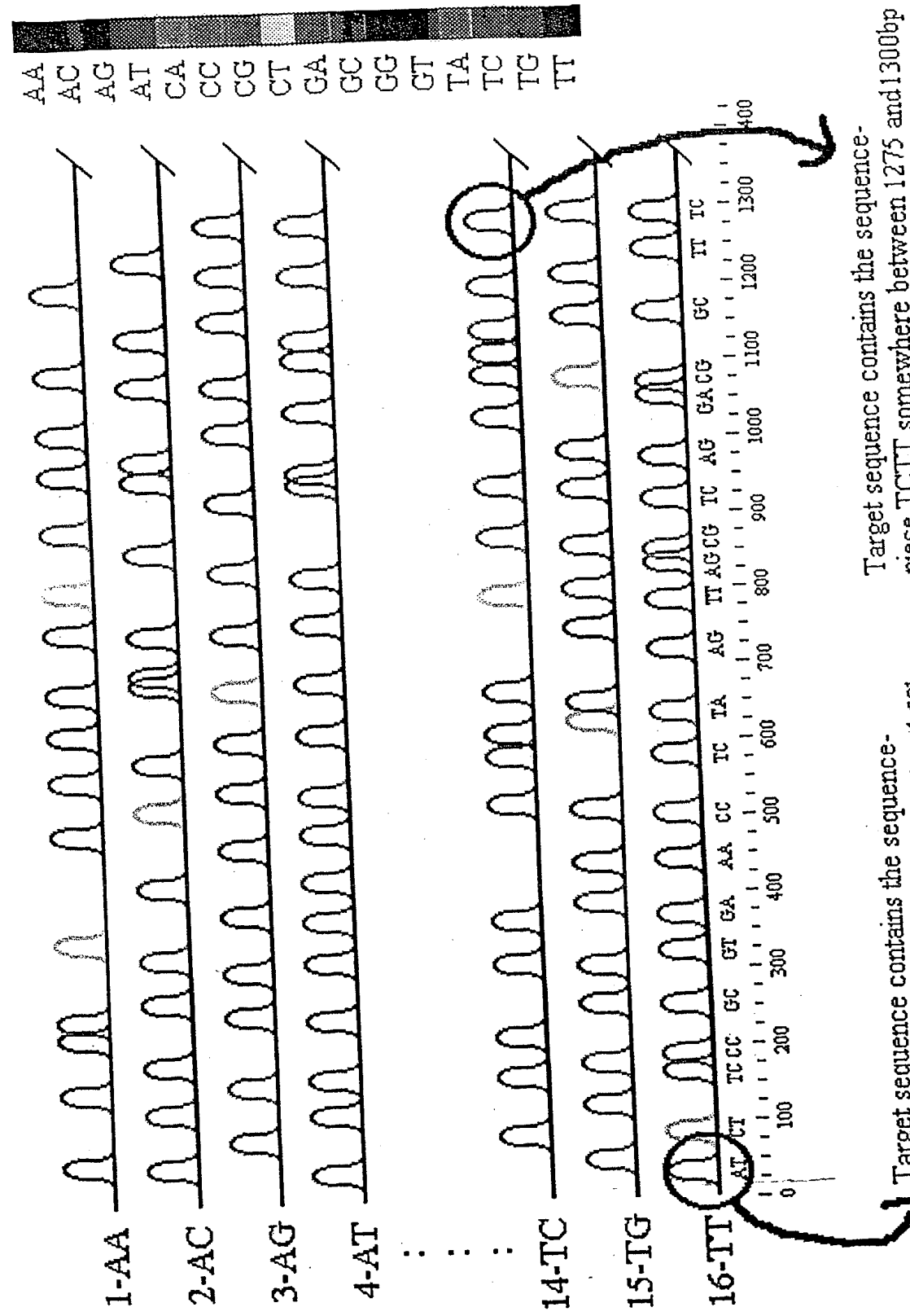

FIG. 12A

FIG. 12B

Preben LEXOW
"SEQUENCING METHOD..."
Q-64884



FIG. 12C



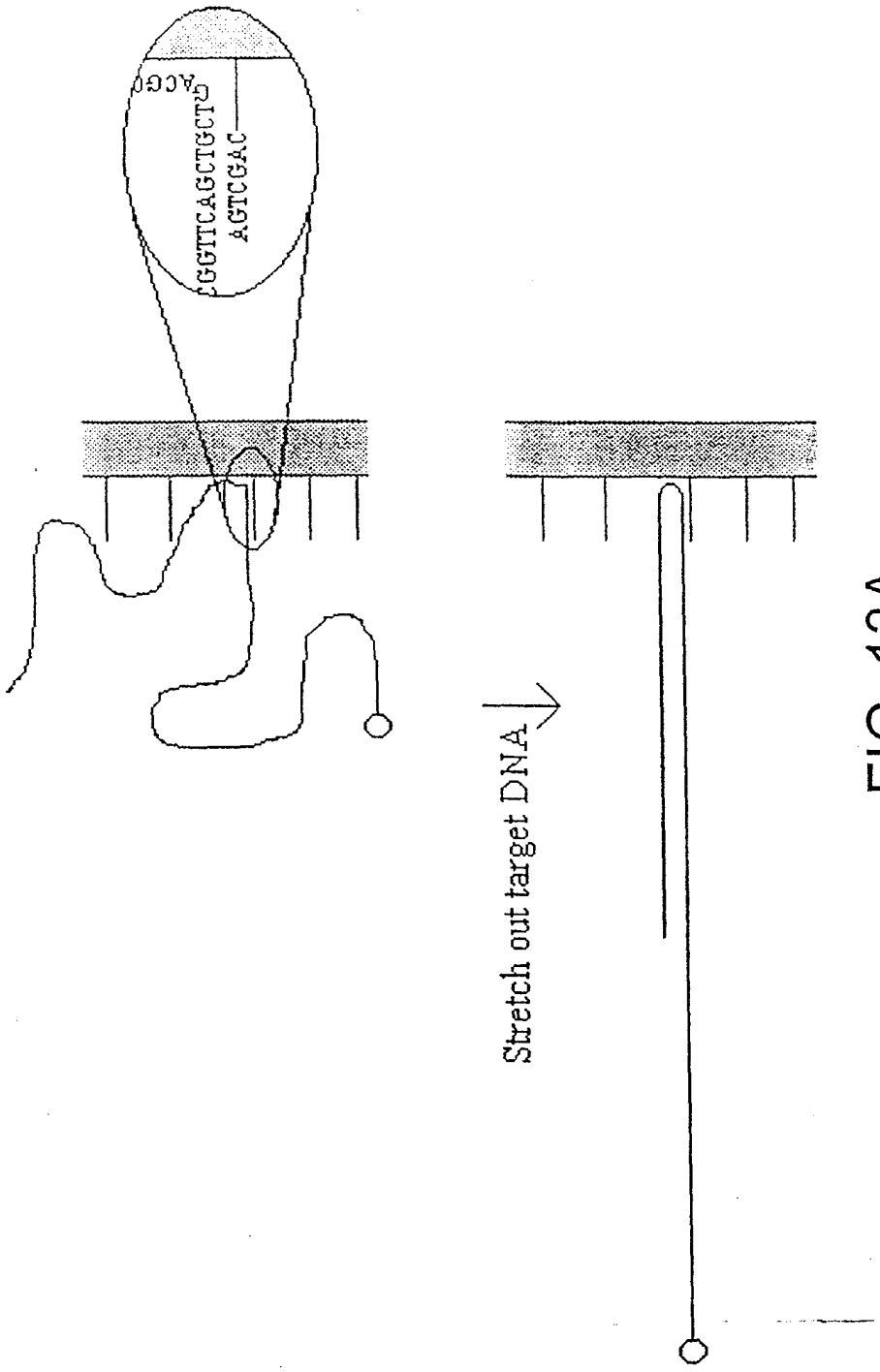



FIG. 13A

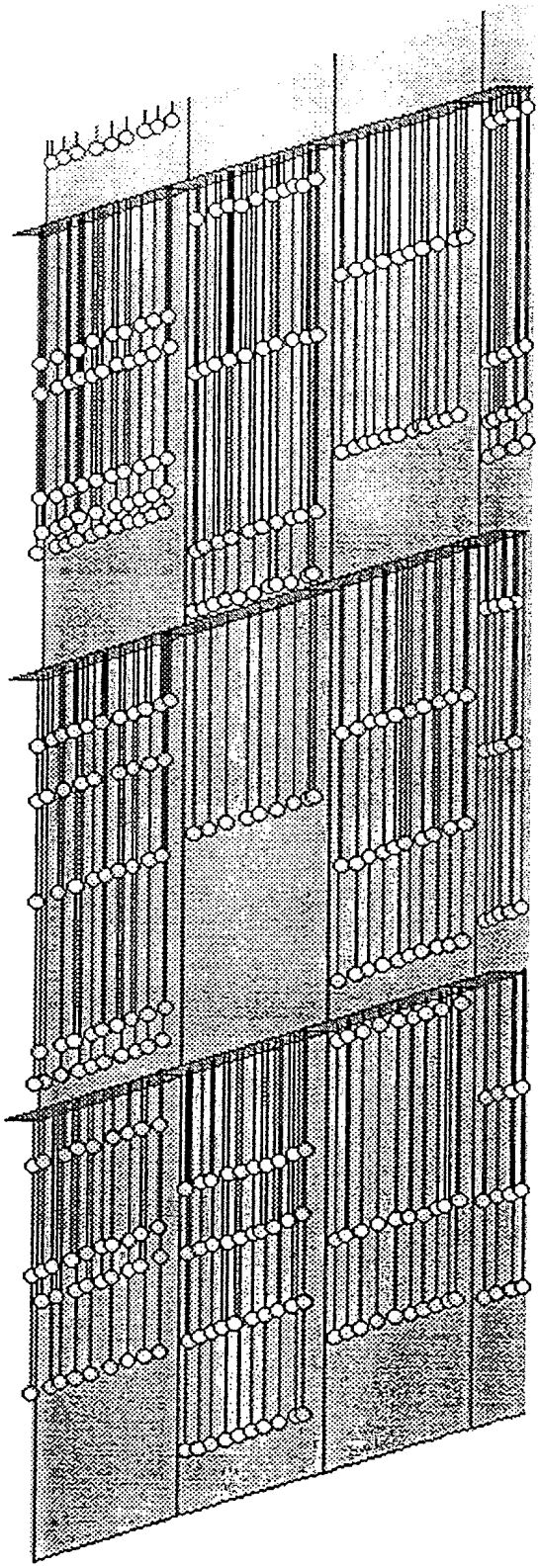


FIG. 13B

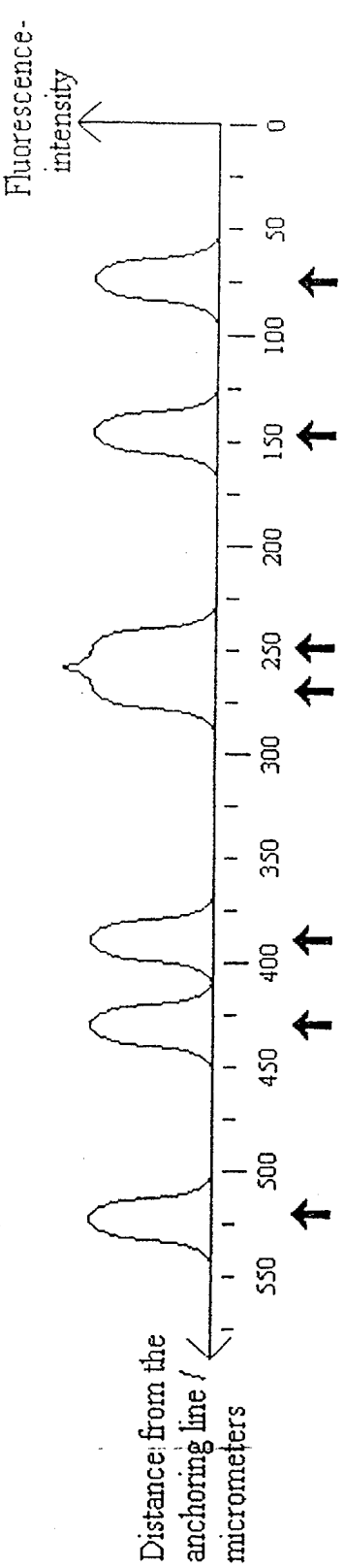



FIG. 13C

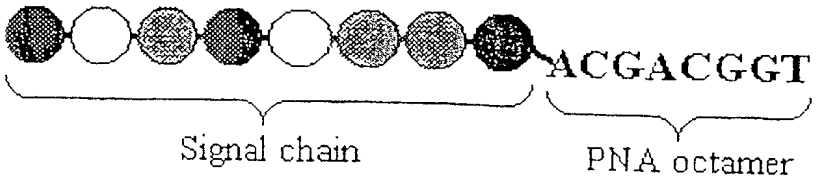



FIG. 14A

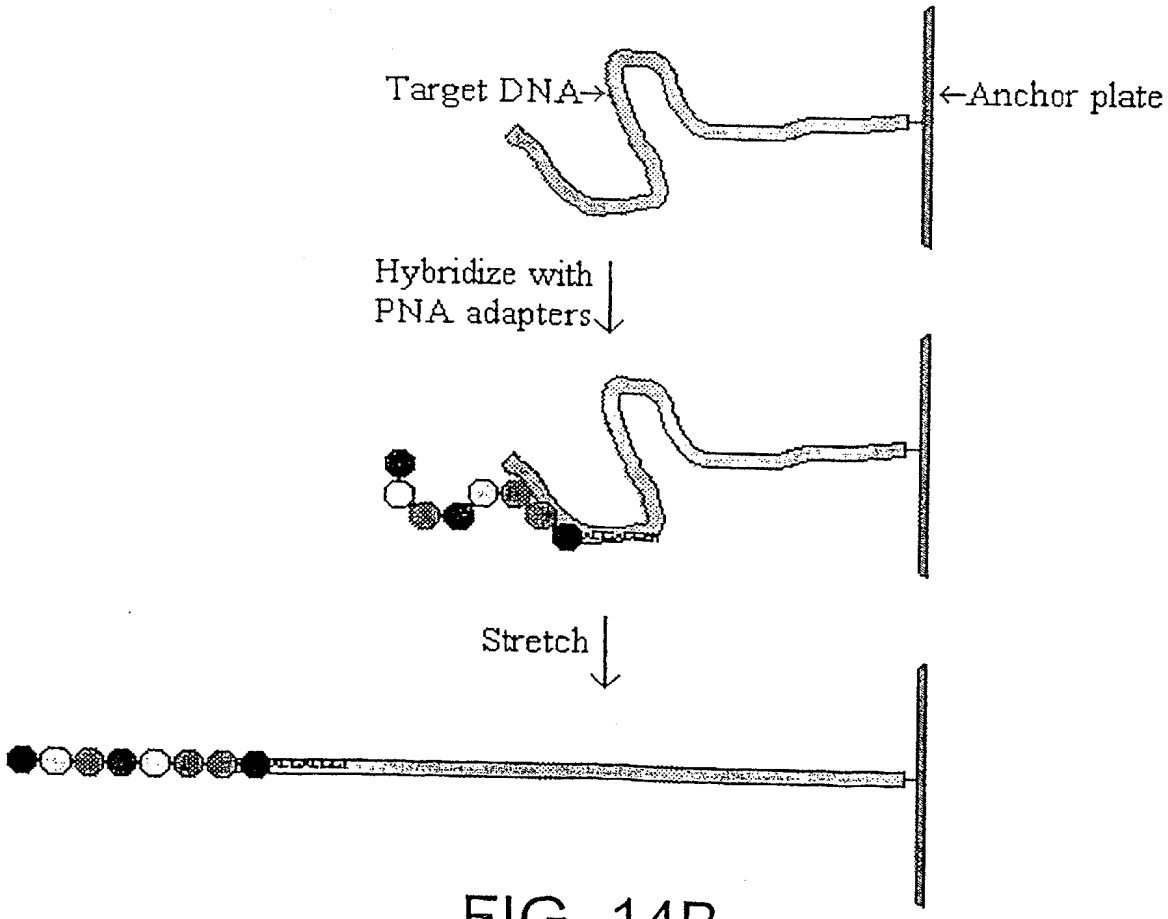



FIG. 14B

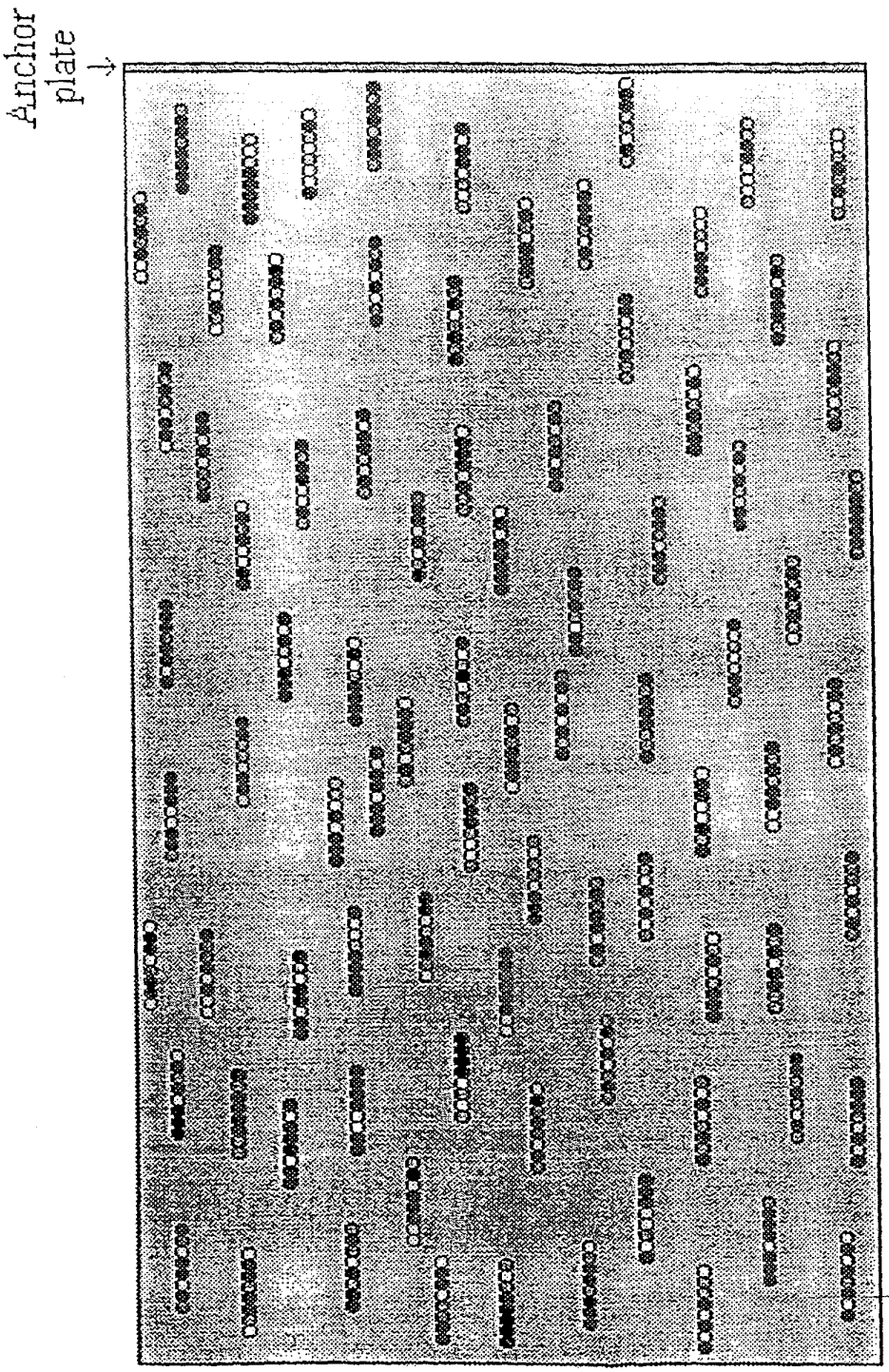



FIG. 14C

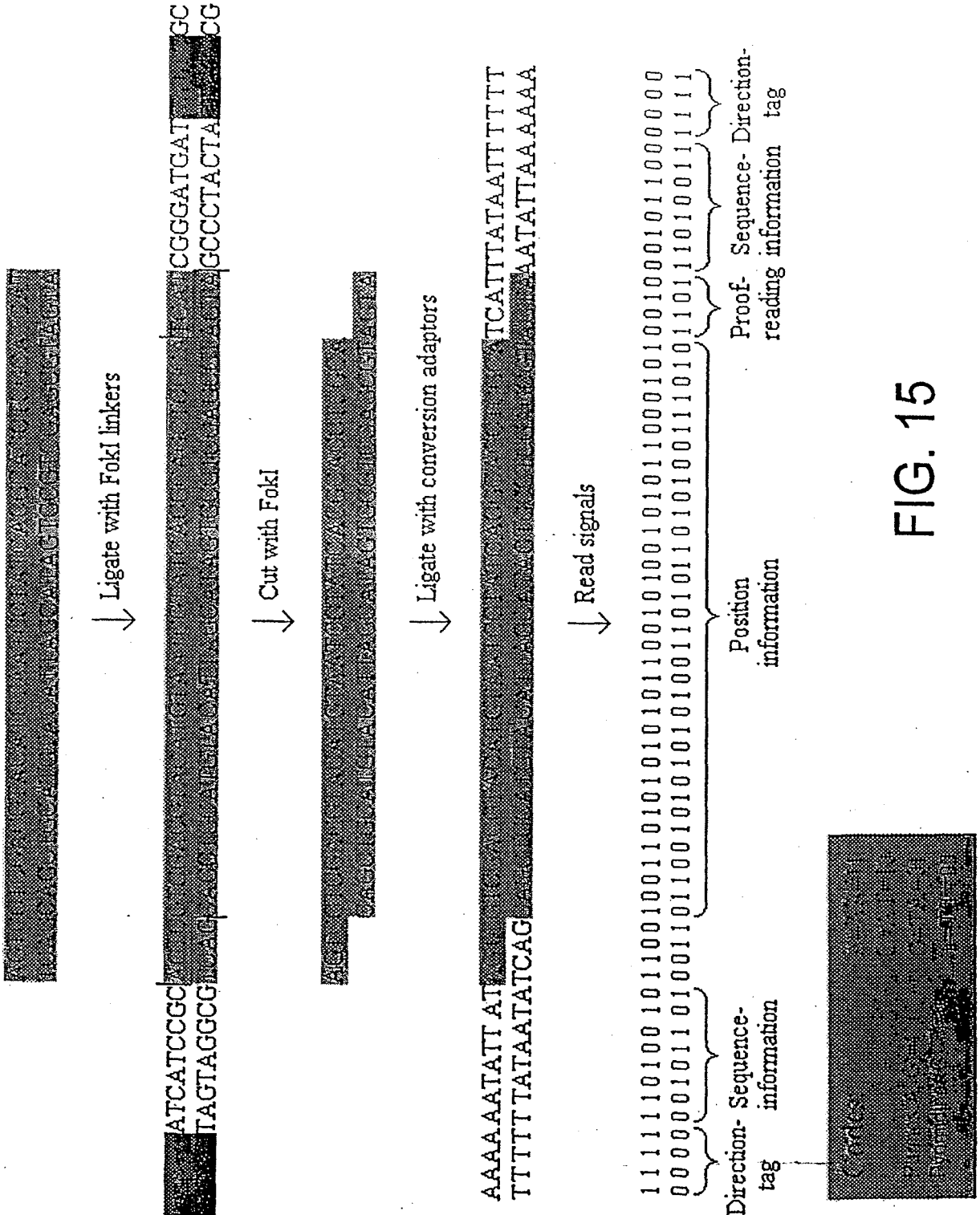

Ligate with FokI linkers
Cut with FokI
Ligate with conversion adaptors
Read signals
Position information
Proof-reading information
Sequence-information
Direction-tag
Sequence-information
Direction-tag

FIG. 15

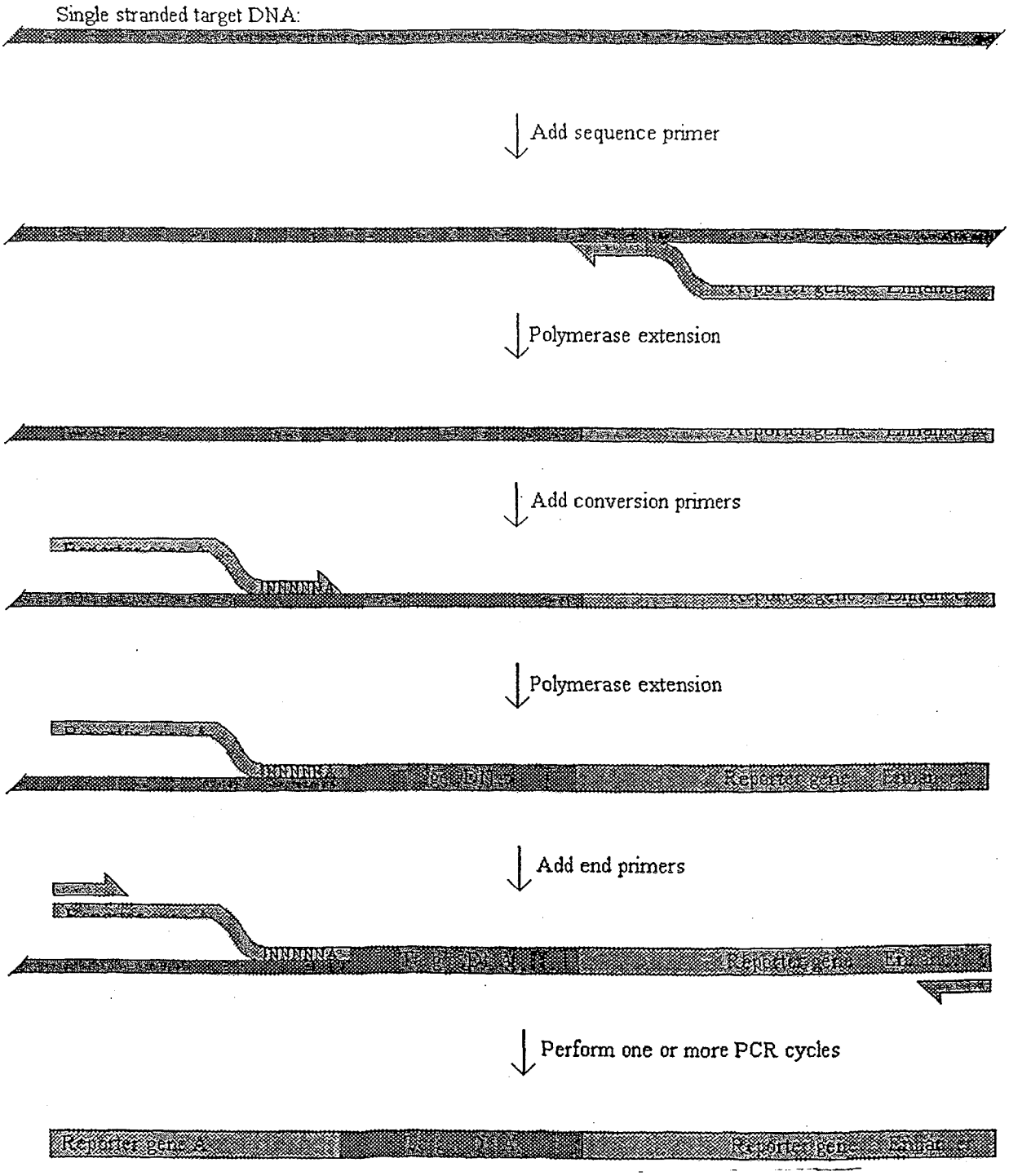

Single stranded target DNA:
Add sequence primer
Polymerase extension
Add conversion primers
Polymerase extension
Add end primers
Perform one or more PCR cycles

FIG. 16A

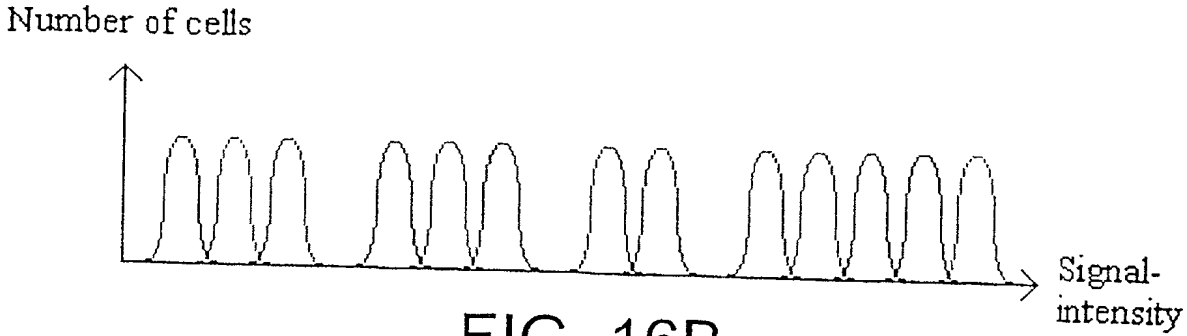



FIG. 16B

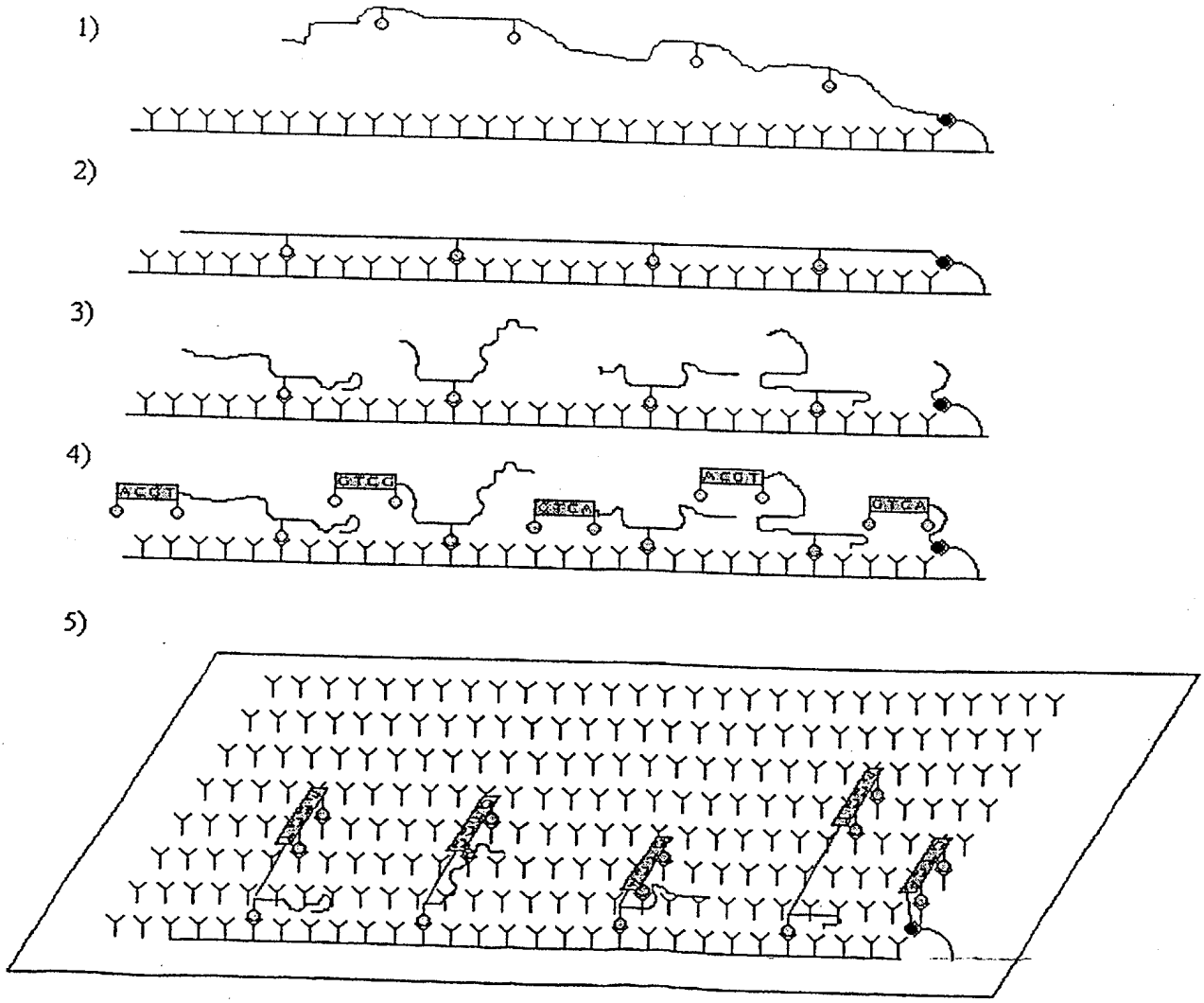



FIG. 18

Preben LEXOW
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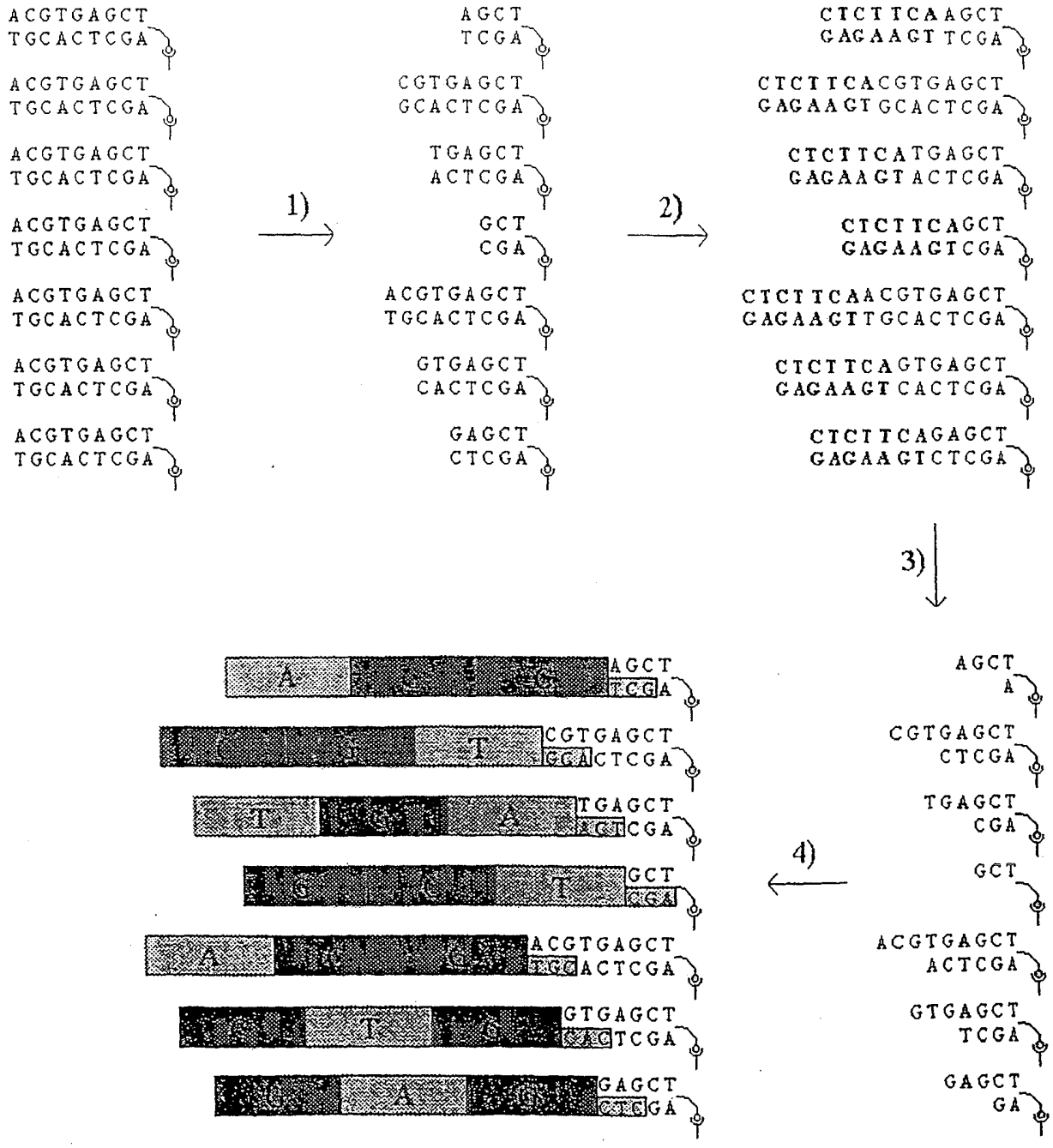



FIG. 17

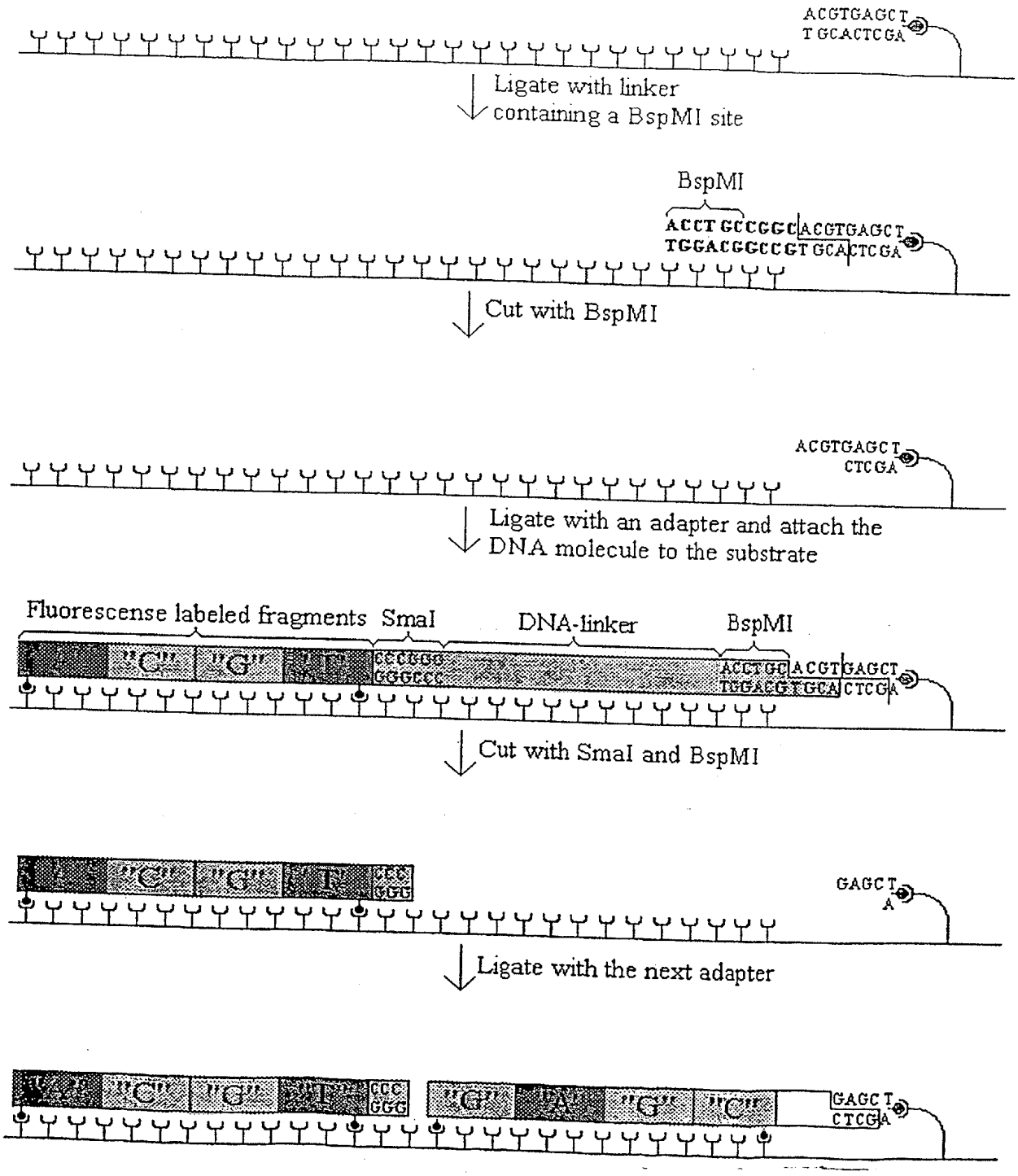

ACGTGAGCT
TGCACTCGA
Ligate with linker
containing a BspMI site
BspMI
ACCTGCCGGCACGTGAGCT
TGGACGGCCGTGCACTCGA
Cut with BspMI
ACGTGAGCT
CTCGA
Ligate with an adapter and attach the
DNA molecule to the substrate
Fluorescense labeled fragments
SmaI
DNA-linker
BspMI
"C"
"G"
CCCGGG
GGGCCC
ACCTGCACGTGAGCT
TGGACGTGCACTCGA
Cut with SmaI and BspMI
GAGCT
A
Ligate with the next adapter
"G"
"A"
"G"
"C"
GAGCT
CTCGA

FIG. 19

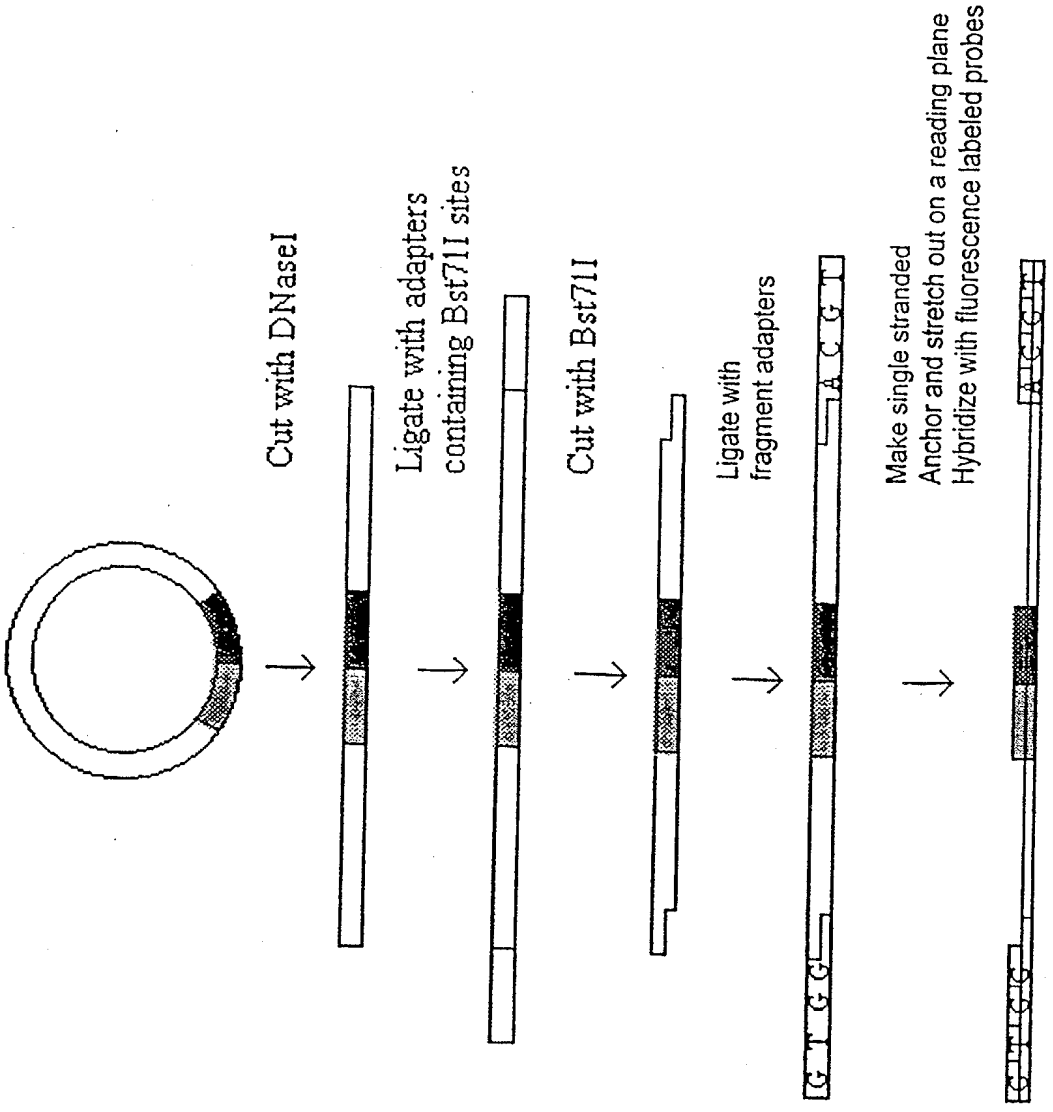

Cut with DNaseI
Ligate with adapters containing Bst711 sites
Cut with Bst711
Ligate with fragment adapters
Make single stranded
Anchor and stretch out on a reading plane
Hybridize with fluorescence labeled probes

FIG. 20

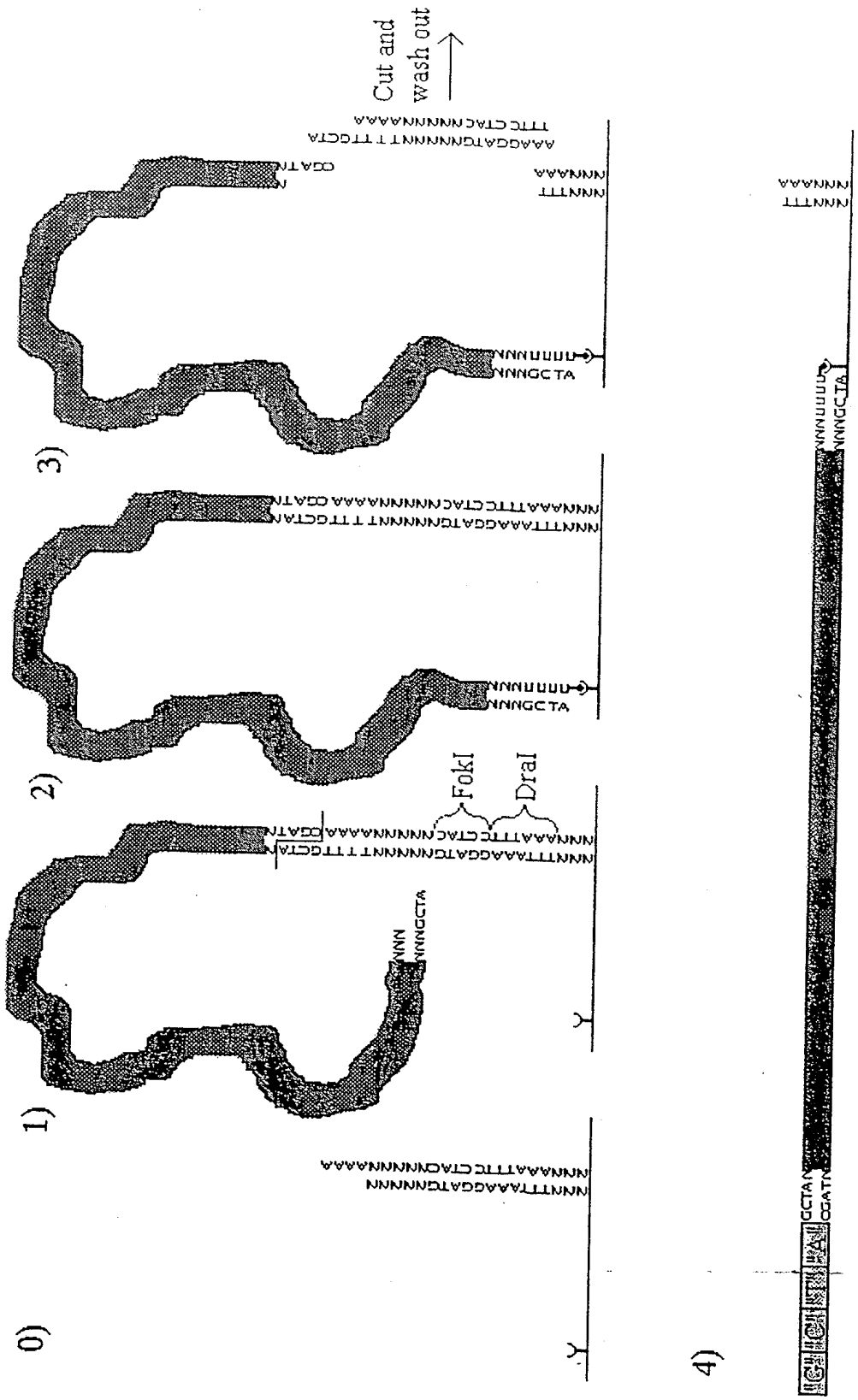



FIG. 21

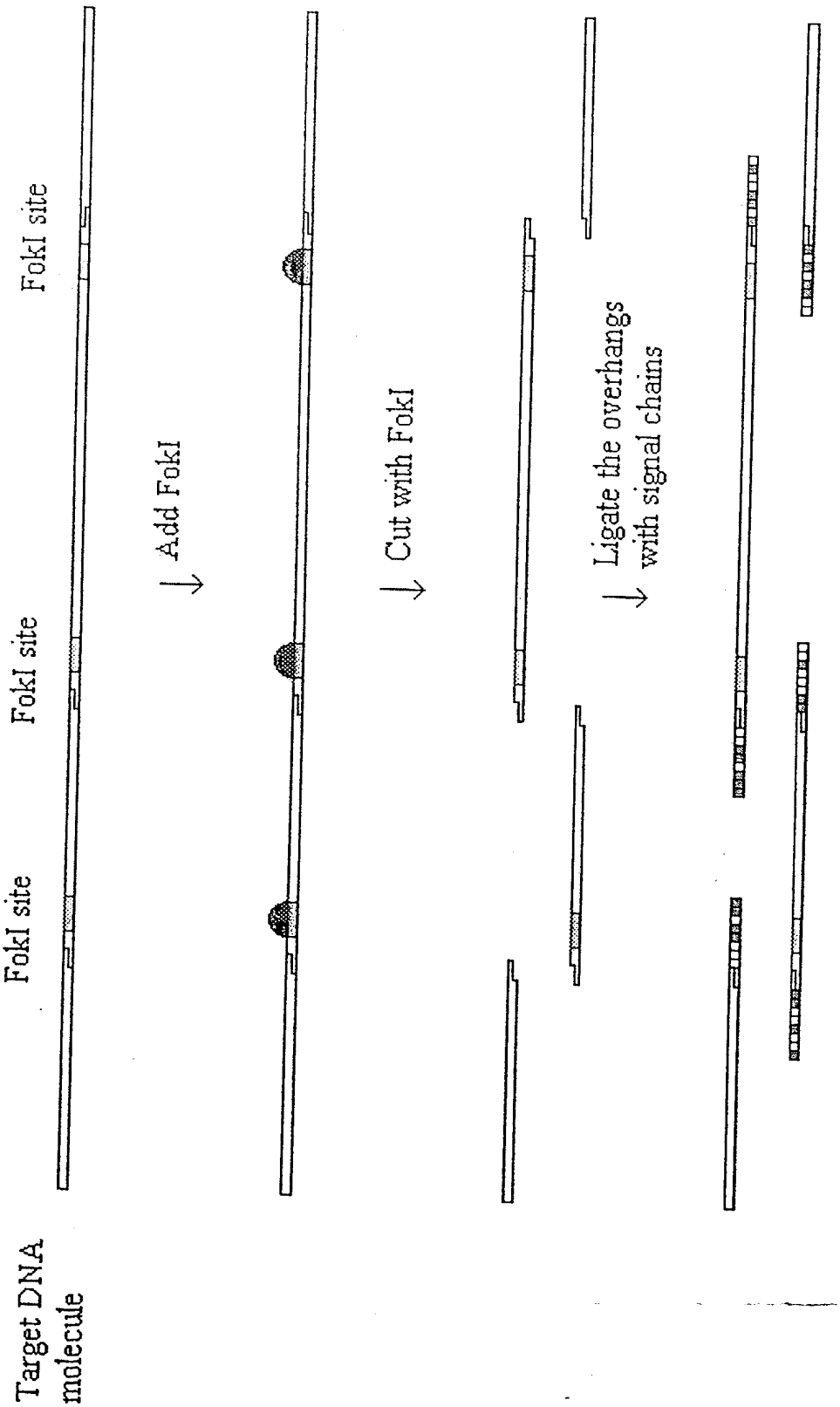

Target DNA molecule
FokI site
FokI site
FokI site
Add FokI
Cut with FokI
Ligate the overhangs with signal chains

FIG. 22

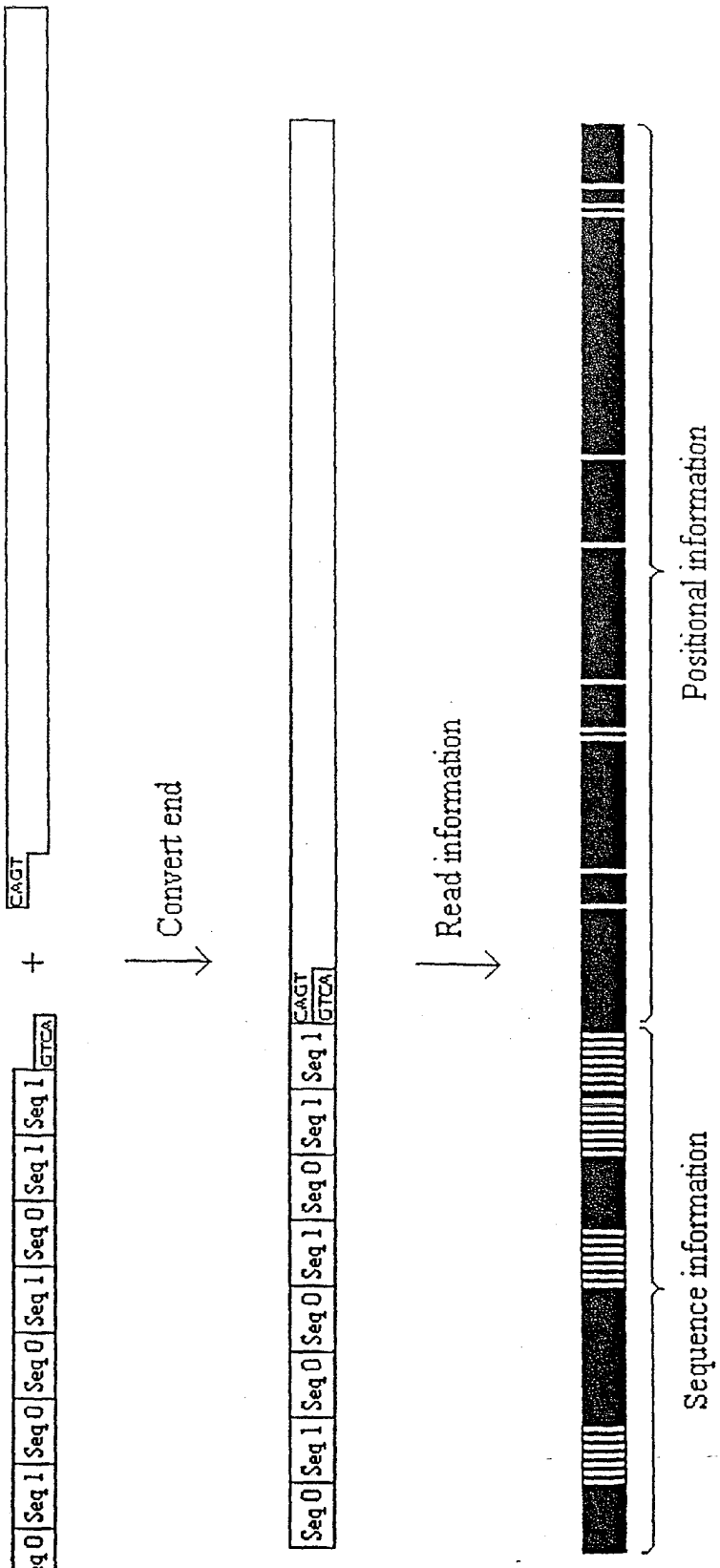
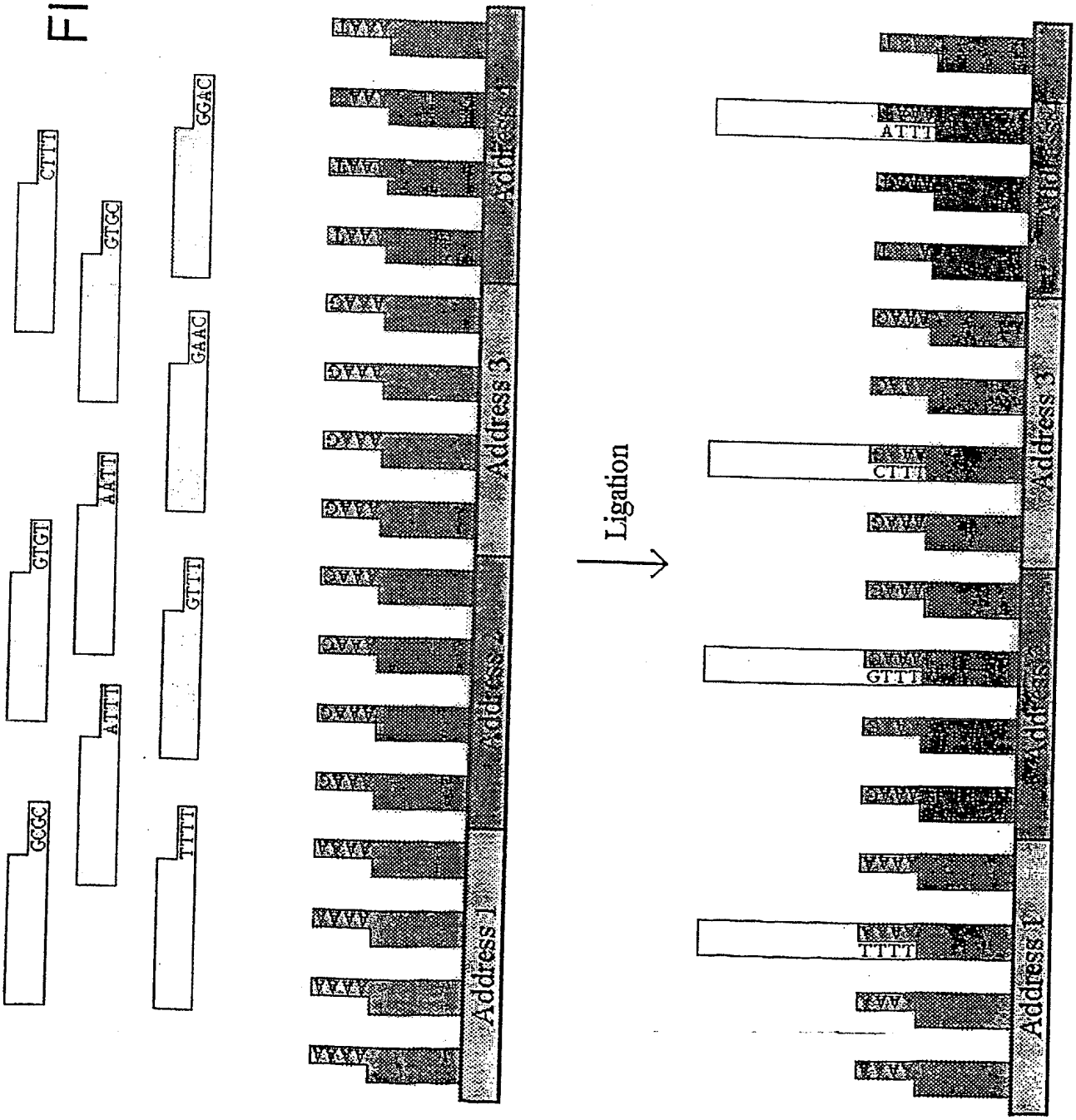

Target DNA:
CAGT
+
Signal chain:
Seq 0 Seq 1 Seq 0 Seq 0 Seq 1 Seq 0 Seq 1 Seq 1 CAGT GTCA
Convert end
Seq 0 Seq 1 Seq 0 Seq 0 Seq 1 Seq 0 Seq 1 Seq 1 CAGT GTCA
Read information
Positional information
Sequence information

FIG. 23

FIG. 24


GGGC
ATTT
TTTT
GTGT
AATT
GTTT
CTTT
GTGC
GAAC
GGAC
Address 1
Address 3
Address 2
Ligation
ATTT
CTTT
GTTT
TTTT
Address 4

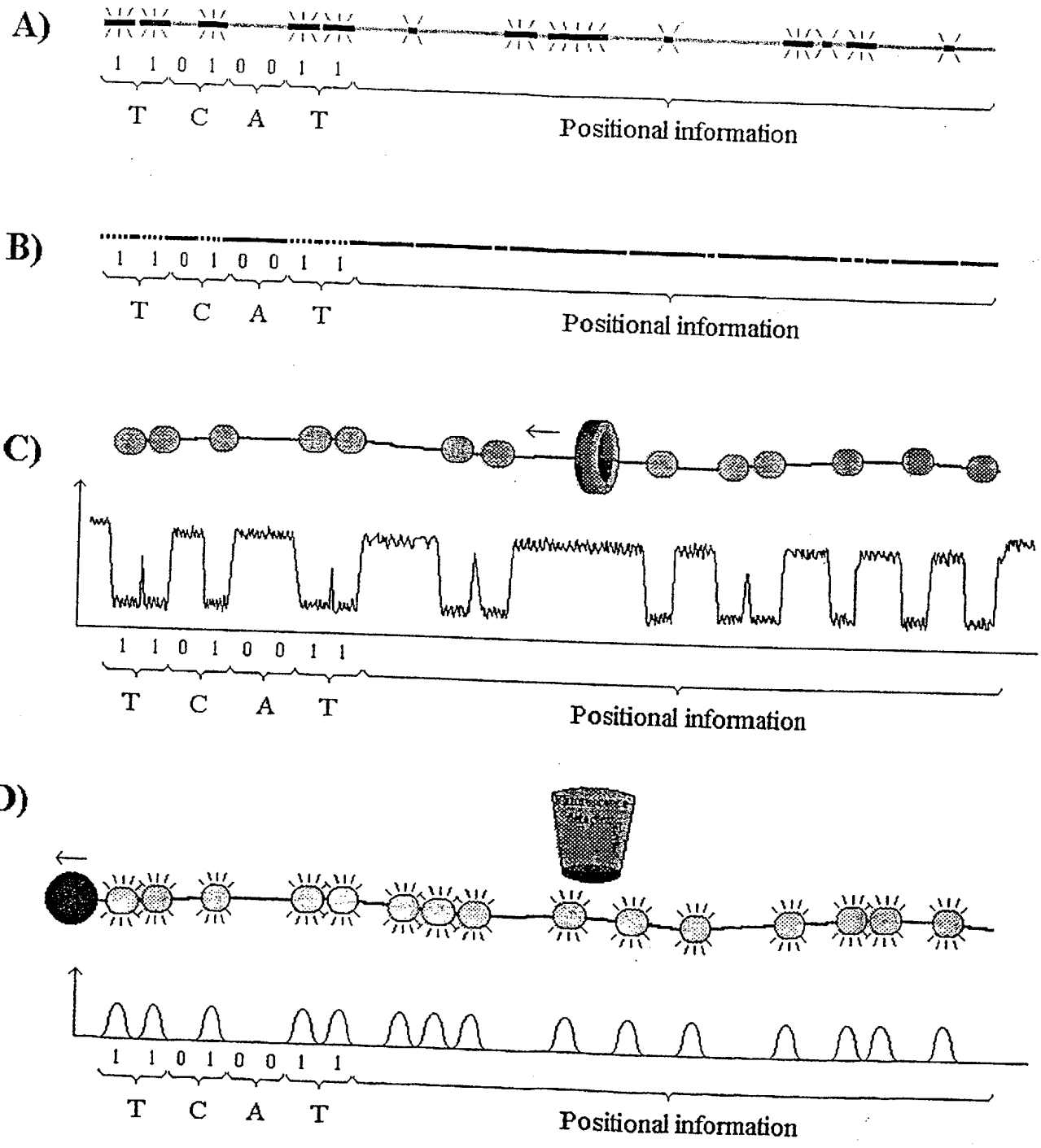

A)
1 1 0 1 0 0 1 1
T C A T
Positional information
B)
1 1 0 1 0 0 1 1
T C A T
Positional information
C)
1 1 0 1 0 0 1 1
T C A T
Positional information
D)
1 1 0 1 0 0 1 1
T C A T
Positional information

FIG. 25